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FUSARIUM RESISTANCE IN WISCONSIN HOLLANDER CABBAGE [1]

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INTRODUCTION

The cabbage-yellows disease, caused by *Fusarium conglutinans* Wr., has been important in the Middle West for the last 3 decades. An investigation of the disease was begun at the Wisconsin Agricultural Experiment Station in 1910 (*3*).[3] From this study it was concluded that the only practical way of controlling the disease was through the development of resistant strains of cabbage (*Brassica oleracea* L.). From two plants of the Danish Ballhead or Hollander variety that remained healthy throughout the season of 1910 on soil thoroughly infested with the yellows organism, continued mass selection was conducted until a resistant strain or variety, known as "Wisconsin Hollander", was secured and introduced for general use in 1916 (*4*). In the early trials Wisconsin Hollander proved to have a high degree of resistance as compared with commercial varieties (*3*). At the end of the season of 1914 the proportion of healthy plants in the F_2 progenies was from 90 to 100 percent. In 1916 the disease was unusually destructive (*4*). Several fields of Wisconsin Hollander showed a high incidence of the disease, and the average percentage of diseased plants for 20 fields was 24.3. The majority of affected plants, however, were but slightly diseased and succeeded in forming heads. This high percentage of diseased plants was probably caused by the severe weather conditions in 1916. Later field observations have likewise indicated that Wisconsin Hollander may show a high percentage of diseased plants under severe conditions of high soil temperature, which is often accompanied by relatively low soil moisture.

Greenhouse tests have shown that Wisconsin Hollander seedlings grown in soil infested with the causal organism differ in degree of resistance (*6*). Resistance to the disease was found to be broken down under high controlled temperatures. Walker and Smith (*9*) compared Wisconsin Hollander with progenies of single plant selections from other varieties at various temperatures in infested soil. They found that the resistance of Wisconsin Hollander to yellows was

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[3] Reference is made by number (italic) to Literature Cited, p. 661.

quite different from that exhibited by homozygous resistant lines developed by Walker (7) and by Walker et al. (8). The latter remained perfectly healthy in infested soil at constant soil temperatures as high as 26° C. Wisconsin Hollander showed quite a high percentage of diseased plants at 20°, with little or no resistance to the disease at 26°. Walker (7) showed that in his homozygous resistant lines resistance was governed by a single dominant gene. He suggested the possibility of hereditary factors being present, which may act quite independently of the main gene for resistance and may modify the expression of the disease. An appreciable percentage of plants of mass-selected varieties, such as Wisconsin Hollander, were suspected of containing such factors.

The object of the investigation reported herein was to study the nature of resistance of Wisconsin Hollander cabbage to the yellows disease in comparison with that displayed by homozygous resistant lines.

MATERIAL AND METHODS

Four types of cabbage host material were used in this study. Wisconsin Hollander seed was secured from various commercial sources. For convenience the individual lots were labeled WH-1, WH-2, WH-3, WH-4, and WH-5.[4] Seeds shown by various field and greenhouse tests to be homozygous resistant and homozygous susceptible were obtained from J. C. Walker. The homozygous resistant lines were as follows: 40-28-A and 40-29-A, selected from All Head Early; 20-29-A, from Jersey Wakefield; 21-30-A, from Copenhagen Market; and 33-31-B, from Danish Ballhead. The homozygous susceptible line used was C-29-A, selected from Copenhagen Market. Two commercial varieties were used, namely, Danish Ballhead, or Hollander, and a Flat Dutch type known as Smith Pride. Both of these lots, when tested, were found to contain from 95 to 100 percent of susceptible individuals.

Methods used in selecting and handling plants for seed propagation have been discussed by previous writers (8). For genetical studies, plants were grown to seed in the greenhouse during the winter, where controlled pollination was practiced throughout. All flowering branches were covered with paper bags to reduce contamination. Field tests were carried out on thoroughly infested soil in Racine and Kenosha Counties, Wis., where similar tests had been made for a number of years. The trial plot was examined at frequent intervals throughout the summer. Each diseased plant was marked with a bamboo stake as a permanent seasonal label.

Greenhouse tests under controlled temperature conditions were carried out mainly in artificially inoculated soil. Inoculum prepared by growing a single-spore isolation of the yellows organism on a mixture of corn meal and sand was thoroughly mixed with soil that had been steamed for a period of 4 hours at a pressure of 15 pounds. Several lots of susceptible seedlings were grown on the inoculated soil in order to ascertain the thorough distribution of the fungus before it was used in the trials reported herein. In a few tests both inoculated soil and naturally infested soil were used. Inoculated soil was found

[4] Although these commercial stocks were presumably derived from the original stock introduced in 1916, it was expected that they would differ from one another after having been propagated for a period of years in the hands of different seedsmen. The difference in degree of resistance is discussed later.

to insure more nearly uniform and more severe tests because of the greater amount and better distribution of the fungus. In naturally infested soil the disease developed less evenly and the test for resistance was prolonged. Wisconsin soil-temperature tanks were employed to maintain constant soil temperatures. The moisture content of the soil was maintained at about 60 percent of the moisture-holding capacity.

FIELD TESTS OF COMMERCIAL WISCONSIN HOLLANDER CABBAGE

The degree of success with Wisconsin Hollander on infested soil is largely dependent on environmental conditions. Under ordinary field conditions a high percentage of plants remain healthy, but in unusually warm midsummer weather 50 percent may show symptoms of the disease (*4*, *7*). During 1929, 1930, and 1931, observations and tests were made in the field. Table 1 shows the results of some of these tests in comparison with commercial Hollander [5] and resistant pure lines. The hot dry weather in the summer of 1931 was very favorable to a severe attack by the yellows organism. In one field in Dane County, near Madison, Wis., 67 percent of the Wisconsin Hollander plants counted were diseased and 19 percent were dead from yellows. In the same field 97 percent of the commercial Hollander plants were diseased and 79 percent were dead from yellows. There were small areas in the Racine commercial field where no diseased plants were found, probably because of incomplete infestation of the soil by the fungus. These two fields were more severely diseased than commercial fields in general, but the data show how Wisconsin Hollander may react under environmental conditions very favorable for the disease.

TABLE 1.—*Occurrence of cabbage yellows in Wisconsin Hollander, in homozygous resistant lines, and in commercial susceptible lines in the field in Wisconsin, 1930 and 1931*

Strain or variety	Location	Year	Total plants	Diseased plants
			Number	*Percent*
WH-5	Experimental plot (Kenosha County).	1930	121	43
40-29-A*	do	1930	157	0
Commercial Hollander	do	1930	289	98
WH-1	Experimental plot (Racine County)	1931	160	52
WH-2	do	1931	30	77
WH-3	do	1931	38	37
WH-4	do	1931	112	97
40-28-A*	do	1931	130	0
Commercial Hollander	do	1931	286	98
Wisconsin Hollander	Commercial field (Dane County)	1931	1,192	67
Commercial Hollander	do	1931	370	97
Wisconsin Hollander	Commercial field (Racine County)	1931	1,430	59

* Homozygous resistant line.

In trials made in 1930 on the Kenosha experimental plot with seed lot WH-5 (table 1) 43 percent of the plants were diseased. The four lots of Wisconsin Hollander tested in 1931 on the Racine experimental plot differed considerably in percentage of diseased plants. Some

[5] Two names, Danish Ballhead and Hollander, are sometimes used interchangeably for this variety. It will be referred to herein as "commercial Hollander."

differences may be expected when seed produced in widely separated localities is used. When seed of a mass-selected variety is produced on soil free from the yellows organism, plants very susceptible to the disease are not eliminated. Seed from continued propagation on such soils would therefore be expected to give a higher percentage of susceptible plants than seed from repeated propagation on soil infested with the yellows organism. Such a history is suspected for the seed lot WH-4, which gave a very high percentage of diseased plants when tested on infested soil.

Under ordinary field conditions the majority of diseased Wisconsin Hollander plants show but incipient yellows and may be classed as slightly diseased. Such plants usually recover and may succeed in forming heads. It is common for such plants to show slight symptoms during the warmest part of the summer and to recover later with the recurrence of cooler weather. Visible symptoms may be confined to one or two of the lower leaves, and as these leaves drop from the plant no further sign of the disease is seen.

F_1 PROGENIES OF SELECTED WISCONSIN HOLLANDER PLANTS

FIELD EXPERIMENTS

For genetical studies, 55 Wisconsin Hollander plants were selected at random, in 1929, from four lots, namely, WH-1, WH-2, WH-3, and WH-4, growing on soil free from the disease organism, and placed in the greenhouse for seed propagation during the winter. Each plant was self-pollinated, and crosses were made with pollen from homozygous resistant and homozygous susceptible lines. Forty three of these plants produced seed.

The F_1 progenies were tested in the field during the summer of 1930. Duplicate plantings of progenies from self-pollination and from the susceptible crosses were made in soil free from the yellows organism, in order to assure material for further genetic studies. On October 29, final notes were taken and all plants of certain progenies were saved and placed in cold storage. The results of field tests with F_1 progenies are presented in table 2. The families are arbitrarily divided into four classes based on the percentage of diseased plants appearing in the progenies from self-pollination.

The progenies from self-pollination showed various degrees of susceptibility and resistance, ranging from 100 percent diseased to 100 percent healthy. The majority of the progenies tested proved to be very high in percentage of plants susceptible to yellows. Twenty-four progenies showed from 75 to 100 percent of the plants diseased (table 2, class 1), and 13 progenies of this class were totally diseased. There was also considerable variation within the individual progenies of this class. In some progenies, such as 8-33s [6] and 8-47s, the plants succumbed completely and comparatively early in the season; in other progenies, such as 8-23s and 8-30s, a large percentage of affected plants were but slightly diseased and some of these recovered; that is, they ceased to show visible symptoms of the disease by the end of the season. The progenies of class 2 and of class 3 showed, respectively, increases in degree of resistance to the disease. A majority of affected plants in these classes were but slightly diseased, and in only

[6] The small letter "s" following a number denotes a progeny from self-pollination.

one progeny was there more than one plant dead of yellows. Two of the progenies tested (class 4) remained entirely healthy throughout the season. Progeny 8-11s appeared to be the most promising in resistance and yield; of the 42 plants of this line, 41 formed heads of good shape and size.

TABLE 2.—*Occurrence of cabbage yellows in the 1930 field tests with F_1 progenies from selected Wisconsin Hollander, commercial Wisconsin Hollander, commercial Hollander, and a homozygous resistant line*

Variety and classification [a]	Parent plant no.	Progenies from self-pollination		Progenies from crosses with—			
				Homozygous susceptible lines		Homozygous resistant lines	
		Total plants	Diseased plants	Total plants	Diseased plants	Total plants	Diseased plants
WH (selection):		*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Class 1 (75 to 100 percent of self-pollinated plants diseased)	8- 1	42	100	22	100		
	8- 2	52	98	50	100	25	0
	8- 3	45	100	48	100	24	0
	8- 5	25	100	49	100		
	8- 7	27	100	42	100		
	8- 8	26	100	36	100	14	0
	8-10	22	100				
	8-12	42	88	54	100	32	0
	8-13	42	95	61	100	25	0
	8-16	29	83	64	98		
	8-23	49	80	60	97	24	0
	8-27	25	100	71	100	26	0
	8-30	54	76	49	80	9	11
	8-32	36	97	45	96	27	0
	8-33	21	100	61	100	26	0
	8-34	25	100	74	92	25	0
	8-36	47	94	60	95	26	4
	8-41	44	100	59	100	29	0
	8-43	31	94	54	100	19	0
	8-45	47	98	54	100	26	0
	8-46	37	100	57	100	31	0
	8-47	49	100	38	100	26	0
	8-51	47	94	47	94	75	0
	8-52	46	100	54	98	68	0
Class 2 (25 to 75 percent of self-pollinated plants diseased)	8-25	21	62	71	99	27	0
	8-38	57	42	64	81	29	0
	8-40	57	61	54	82	26	0
	8-42	12	50	81	65	26	0
	8-53	37	32	59	93	58	0
Class 3 (1 to 25 percent of self-pollinated plants diseased)	8- 4	47	19	49	65		
	8- 9	26	27	41	80	27	0
	8-14	36	8	48	38	25	0
	8-15	34	18	66	67	26	0
	8-17	51	4				
	8-19	50	18	54	96	27	0
	8-26	58	14	53	74		
	8-28	47	6	61	23	26	0
	8-37	17	12	64	58	25	0
	8-39	63	21	29	79	26	0
Class 4 (no disease in self-pollinated plants)	8-11	42	0	41	61	28	0
	8-49	26	0	73	49	27	0
WH-5 (commercial)		121	43				
Commercial Hollander		289	98				
Homozygous resistant		157	0				

[a] For convenience the parent plants are arbitrarily divided into four classes based on the percentage of diseased plants appearing in the progenies from self-pollination.

For comparison with these progenies from self-pollination of selected Wisconsin Hollander plants, Wisconsin Hollander (WH-5), commercial Hollander, and a homozygous resistant line were also tested. Most of the plants of the commercial Hollander were dead of yellows at the end of the season. Forty-three percent of the commercial Wisconsin Hollander were diseased. Of these, 21 percent

were severely diseased or dead and 22 percent but slightly diseased. No symptoms of the disease appeared in plants of the homozygous resistant line.

Except 2 plants of 987 tested, the progenies from crosses of selected Wisconsin Hollander × homozygous resistant plants remained perfectly healthy throughout the season. In view of the fact that the pistillate mother plants of the two progenies in question gave very susceptible progenies from self-pollination, it is suspected that the two diseased plants were the result of chance self-pollination. The progenies from crosses with homozygous susceptible plants were in general very susceptible to the disease. Not only were the percentages of diseased plants high, but the majority of such plants were dead of yellows or severely diseased.

Certain F_1 progenies were tested in the field in 1931. Homozygous resistant plants and commercial Hollander were also planted for comparison. Environmental conditions were more favorable for the disease during this season and the percentage of diseased plants was much higher throughout. Progenies from crosses with homozygous susceptible lines, which had exhibited some resistance in 1930 (table 2, class 3), showed a considerable increase in percentage of diseased plants in 1931. The homozygous resistant line and the commercial Hollander reacted identically as in 1930 (table 3).

TABLE 3.—*Occurrence of cabbage yellows in 1931 field tests of F_1 progenies from selected Wisconsin Hollander, commercial Hollander, and a homozygous resistant strain*

[Data represent the combined results of plantings on two separate dates]

Parent plant no. or variety	Progenies from self-pollination		Progenies from crosses with homozygous susceptible plants	
	Total plants	Diseased plants	Total plants	Diseased plants
Wisconsin Hollander (selection):	*Number*	*Percent*	*Number*	*Percent*
8-4	29	72	60	93
8-11	48	8	39	62
8-14	45	62	44	75
8-23	45	93	15	100
8-28	22	50	34	85
8-30	31	87	70	89
8-33	87	100	67	100
8-37	42	55	28	86
8-39	53	53	65	91
8-40	24	88	15	100
8-47	22	100	56	100
8-53	20	70	61	95
Commercial Hollander	286	98		
Homozygous resistant	130	0		

GREENHOUSE EXPERIMENTS

REACTION AT RELATIVELY HIGH SOIL TEMPERATURES

Studies with Wisconsin Hollander under conditions of controlled soil temperature were planned to supplement the field tests. The most resistant F_1 progenies from self-pollinated Wisconsin Hollander plants and from crosses with susceptible plants were tested in October and November in comparison with homozygous resistant and homozygous susceptible lines (table 4). The tests were continued for 25

to 29 days at a soil temperature of about 22° C. and an air temperature of about 20° C. All the F_1 progenies from self-pollination showed a high percentage of diseased plants and some were totally diseased. The majority of diseased plants in these progenies were dead of yellows at the end of the test. The 8-4s strain, however, showed more resistance than in the field trials. The progenies from the susceptible crosses exhibited very few plants free from the disease, and most of the diseased plants were dead at the end of the experiment. In these tests, the susceptible cross with 8-49, which showed some resistance in the 1930 field trials (table 2), proved to be more susceptible than the susceptible cross with 8-11. The commercial Hollander and the homozygous susceptible strain tested succumbed completely to the disease, whereas the homozygous resistant strain remained perfectly healthy.

A similar experiment, with commercial Wisconsin Hollander and the most resistant F_1 progenies from self-pollination of selected Wis-

FIGURE 1.—Comparison of the rate of disease development in three susceptible F_1 progenies from Wisconsin Hollander plants grown on infested soil at 20° to 22° C. for 21 days. Many of the plants of 8-46s and 8-53s show incipient yellows, and all the plants of 8-47s have succumbed to the disease. At the end of 36 days most of the plants of 8-46s and 8-53s were dead of yellows.

consin Hollander plants, was conducted in May, when the air temperature was slightly higher than in the previous tests. In this test the progenies showed a higher percentage of diseased plants than in the first experiment.

The more susceptible F_1 progenies from self-pollination when similarly tested showed little or no resistance. All plants in some progenies died comparatively early, whereas plants in other progenies were uniformly slower to succumb. Figure 1 illustrates the differences in results obtained from progenies showing slightly different reactions to the disease.

In another experiment four F_1 progenies that had previously shown various rates of disease development in field and greenhouse were grown at a series of constant soil temperatures. The four strains represented the most susceptible progeny (8-33s), two intermediate progenies (8-46s and 8-39s), and the most resistant progeny (8-11s) from self-pollination of Wisconsin Hollander plants. The percentage

of diseased plants at various intervals and the condition of the plants at the end of the experiment are shown in table 5.

TABLE 4.—*Occurrence of cabbage yellows in the most resistant F_1 progenies from selected Wisconsin Hollander, commercial Hollander, a homozygous resistant line, and a homozygous susceptible line, when grown at a constant soil temperature of 22° C. in inoculated soil*

Parent plant no. or variety	Progenies from self-pollination		Progenies from susceptible crosses	
	Total plants	Diseased plants	Total plants	Diseased plants
Wisconsin Hollander (selection):	*Number*	*Percent*	*Number*	*Percent*
8-4	38	53	47	98
8-9	40	80		
8-11	40	73	30	90
8-14	40	100	35	100
8-15	40	93	45	100
8-17	40	90	33	100
8-19	40	93		
8-26	40	80	45	98
8-28	40	78	45	100
8-37	40	100	37	100
8-39	40	100		
8-49			40	98
Commercial Hollander	30	100		
Homozygous susceptible	47	100		
Homozygous resistant	48	0		

TABLE 5.—*Development of cabbage yellows in certain F_1 progenies from selected Wisconsin Hollander plants when grown in inoculated soil at various constant soil temperatures*

Soil temperature	Strain no.	Total plants	Plants diseased after—						Plants in indicated condition after 21 days			
			6 days	8 days	11 days	14 days	19 days	21 days	Healthy	Slightly diseased	Severely diseased	Dead of yellows
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
16° C.	8-33s	39	0	0	21	33	61	80	20	29	51	0
	8-46s	43	0	0	0	9	14	51	49	42	9	0
	8-39s	37	0	0	0	5	8	46	54	35	11	0
	8-11s	27	0	0	0	4	4	11	89	11	0	0
18° C.	8-33s	37	0	0	54	84	100	100	0	0	49	51
	8-46s	45	0	0	18	36	58	100	0	53	47	0
	8-39s	37	0	0	19	32	76	88	12	45	40	3
	8-11s	28	0	0	0	11	11	32	68	21	11	0
20° C.	8-33s	39	0	18	77	100	100	100	0	0	8	92
	8-46s	45	0	0	31	80	91	100	0	13	25	62
	8-39s	38	0	0	47	87	95	100	0	8	29	63
	8-11s	26	0	0	19	42	58	62	38	31	19	12
22° C.	8-33s	36	8	70	100	100	100	100	0	0	0	100
	8-46s	44	0	0	52	95	100	100	0	2	18	80
	8-39s	37	0	0	70	73	100	100	0	3	16	81
	8-11s	31	0	0	16	58	74	74	26	41	23	10
24° C.	8-33s	46	22	94	100	100	100	100	0	0	0	100
	8-46s	42	0	17	93	100	100	100	0	0	2	98
	8-39s	40	0	15	93	100	100	100	0	0	3	97
	8-11s	27	0	11	78	93	96	100	0	7	19	74
26° C.	8-33s	43	28	88	100	100	100	100	0	0	0	100
	8-46s	45	0	18	100	100	100	100	0	0	0	100
	8-39s	35	0	37	100	100	100	100	0	0	0	100
	8-11s	25	0	20	92	96	100	100	0	4	4	92

Both in percentage of plants diseased and in the condition of the diseased plants, the severity of the disease increased in all strains with the rise in soil temperature. Even the most resistant strain became totally diseased at temperatures above 22° C. This strain (8-11s) showed a relatively high degree of resistance to yellows at 16° and 18°, the majority of affected plants at these temperatures being but slightly diseased. The most susceptible strain (8-33s), however, showed little or no resistance to yellows at the soil temperatures maintained. The relative degrees of resistance of the four strains at the end of 14 days are shown in figure 2. At the end of the experiment the relative degrees of resistance were apparent only at the lower temperatures. At the higher temperatures all strains were totally diseased. The incubation period was consistently shorter in the more susceptible strains. The homozygous resistant lines remained completely healthy at all temperatures from 16° to 26°.

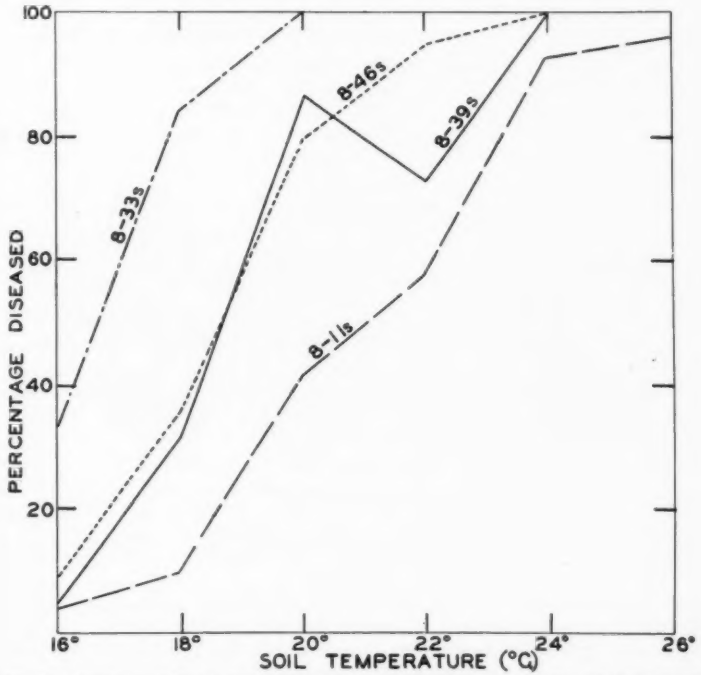

FIGURE 2.—Comparison of four F_1 progenies from Wisconsin Hollander plants at the end of 14 days when grown in inoculated soils at various constant soil temperatures. (See table 7.)

The reactions of 8-33s, 8-53s, 8-11s, and homozygous resistant 33-31-B, under similar conditions, are shown in figure 3, demonstrating the wide variation in rate and degree of disease development in the F_1 progenies from Wisconsin Hollander plants.

The results of the field tests show that the progenies from self-pollination ranged from 100 percent diseased to 100 percent healthy.

Some of the mother plants apparently transmitted little resistance to their progenies, and these were probably of the class in the Wisconsin Hollander variety which become severely diseased or succumb under environmental conditions favorable for the disease. The progenies of several mother plants contained individuals that were but slightly affected by the disease and showed a tendency to recover. The progenies of a small percentage of the plants exhibited a high degree of resistance under severe field conditions, and the majority of the diseased plants were but slightly affected. However, constant soil temperatures at 22° to 24° C. served to inhibit the expression of this resistance, whereas the resistance of the homozygous resistant lines, attributed by Walker (7) to the gene *R*, remained completely stable. This physiological test serves to distinguish between the resistance in Wisconsin Hollander and that in the homozygous resistant lines described by Walker.

Gradations in degree of disease development among the F_1 progenies were quite evident in the greenhouse tests. It was also

FIGURE 3.—Comparison of three F_1 progenies from self-pollinated Wisconsin Hollander plants and a homozygous resistant line, when grown in inoculated soil at a constant soil temperature of 22° C. for 30 days. All plants of strain 8-33s are dead; all plants of strain 8-53s are diseased, and 3 have partially recovered in strain 8-11s, 2 plants have remained healthy throughout, and 7 have shown slight symptoms and are recovering. The homozygous resistant strain, 33-31-B, has remained healthy. Each can contained 15 plants.

distinctly noticeable that reaction of individuals within any one F_1 progeny was more uniform than in the commercial Wisconsin Hollander variety. The practice of self-pollination tends to increase the uniformity of the degree of resistance or susceptibility in the individuals of a given F_1 progeny as compared with that of the Wisconsin Hollander variety in general.

The degree of resistance of the mother plants, as displayed in tests with progenies from self-pollination, is considerably diminished in progenies from crosses between those mother plants and homozygous susceptible plants. When Wisconsin Hollander plants were crossed with plants homozygous for the main gene for resistance the dominance of the latter in the F_1 hybrids was complete. Walker (7) discussed resistance in homozygous resistant and homozygous susceptible lines on the basis of plants falling into two discontinuous classes. In Wisconsin Hollander, however, many intermediate classes or degrees of resistance are found. These classes differ from one another not only in the percentage of plants susceptible to the

disease but also in the severity of the disease in such plants. If the resistance exhibited by the Wisconsin Hollander variety were controlled by a single Mendelian factor, the progeny from self-pollination of a heterozygous plant would be expected to give 3 resistant plants to 1 susceptible, and the progeny from a heterozygous × susceptible cross should give 1 resistant to 1 susceptible plant. None of the F_1 progenies tested over a period of 2 years gave susceptible and resistant plants in numbers approximating these ratios. The resistance of Wisconsin Hollander, therefore, is apparently the result of factors genetically different from the single Mendelian factor of the homozygous resistant lines described by Walker (7).

Reaction of Susceptible Progenies at Low Soil Temperatures

In the greenhouse studies it was observed that in certain very susceptible F_1 progenies from self-pollinated Wisconsin Hollander plants the development of disease was more rapid at low temperatures than had heretofore been observed. Experiments were planned to study this question more critically. A coverless Wisconsin soil-temperature tank was used in these experiments. A low temperature was maintained in the tank by a constant stream of cold water. The air temperature was quite constant at 22° to 24° C. Small variations of soil temperature between cans and within each can were obtained by placing the cans at various depths in the tank. The cans were placed in five rows across the tank. The soil level in the first row of cans was slightly lower than the water level; that of each succeeding row was raised in successive increments of 2 inches. The last row of cans was placed on top of the tank. With such an arrangement constant soil temperatures were maintained at a small increase in temperature in each ascending row. In each can there was a slight variation in temperature at different depths of the soil. This variation more closely simulated soil-temperature conditions in the field than does the ordinary tank method, in which the whole can is at the same temperature.

In the first experiment, progenies 8-33s and 8-46s were studied. In the field trials (table 2) both strains had shown 100 percent of the plants diseased, but the plants of strain 8-33s were more severely diseased than those of strain 8-46s. The soil temperature varied from 13.5° C. in the first row of cans (A) to 20.1° in the fifth row (E). The percentage of diseased plants was ascertained at various intervals over a period of 23 days (table 6). At the three lower temperatures there appeared to be a distinct difference between the two strains in the rate of development of the disease. Progeny 8-33s showed a high percentage of diseased plants at all temperatures, and the few plants showing but slight symptoms were confined to the cans of the two lower temperatures. Progeny 8-46s showed no symptoms in row A and but slight symptoms in rows B and C.

In the second experiment a homozygous resistant strain (33-31-B) was grown in comparison with 8-33s and 8-46s, and the test was continued for 42 days. Lower temperatures were maintained to find the critical temperature at which symptoms of the disease appear in the very susceptible strain 8-33s. Temperatures of the surface soil were noted as well as those at depths of 1 and 3 inches. The results (table 7 and fig. 4) further indicate a distinct difference in the rate of

disease development. Strain 8-33s showed disease symptoms at all temperatures. At the lowest temperature (row A), however, the symptoms were slight and confined to the lower leaves. These plants were not stunted by the disease. No symptoms of disease appeared in strain 8-46s at 10° to 12° C. Slight symptoms appeared at slightly higher temperatures (table 7, B and C). At the end of the experiment some of the plants in row C had ceased to show external symptoms of the disease as a result of abscission of the lower leaves. The homozygous resistant strain remained healthy at all temperatures of the experiment.

TABLE 6.—*Development of cabbage yellows in two F_1 progenies from Wisconsin Hollander plants of different degrees of susceptibility when grown in inoculated soil at various soil temperatures*

[20 plants of 8-33s and 12 plants of 8-46s were grown in each row]

Row	Soil temperature at the depth of—		Strain no.	Plants diseased after—					Plants in indicated condition after 23 days		
	3 inches	1 inch		10 days	12 days	15 days	19 days	23 days	Slightly diseased	Severely diseased	Dead of yellows
	°C.	°C.		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A	13.5	13.9	8-33s	0	0	10	45	70	35	35	0
			8-46s	0	0	0	0	0	0	0	0
B	13.9	14.6	8-33s	10	45	60	100	100	10	90	0
			8-46s	0	0	0	17	33	33	0	0
C	14.5	15.6	8-33s	75	100	100	100	100	0	5	95
			8-46s	0	8	8	17	17	17	0	0
D	16.0	17.3	8-33s	100	100	100	100	100	0	0	100
			8-46s	17	75	83	92	100	8	33	59
E	20.1	20.0	8-33s	100	100	100	100	100	0	0	100
			8-46s	8	33	92	100	100	0	8	92

TABLE 7.—*Development of cabbage yellows in two susceptible F_1 progenies from self-pollinated Wisconsin Hollander plants and in a homozygous resistant strain when grown in inoculated soil at various low soil temperatures*

[15 plants of each strain were grown in each row]

Row	Soil temperature at the depth of—			Strain no.	Plants diseased after—						Plants in indicated condition after 42 days		
	3 inches	1 inch	¼ inch		8 days	14 days	21 days	29 days	38 days	42 days	Slightly diseased	Severely diseased	Dead of yellows
	°C.	°C.	°C.		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A	10.9	11.9	12.3	8-33s	0	0	0	20	33	47	47	0	0
				8-46s	0	0	0	0	0	0	0	0	0
				33-31-B	0	0	0	0	0	0	0	0	0
B	11.8	13.4	14.3	8-33s	0	0	53	80	87	100	27	73	0
				8-46s	0	0	0	33	47	80	80	0	0
				33-31-B	0	0	0	0	0	0	0	0	0
C	13.1	14.8	15.6	8-33s	0	27	100	100	100	100	0	27	73
				8-46s	0	0	20	40	60	67	60	7	0
				33-31-B	0	0	0	0	0	0	0	0	0
D	15.5	17.0	17.2	8-33s	13	93	100	100	100	100	0	0	100
				8-46s	0	80	100	100	100	100	0	7	93
				33-31-B	0	0	0	0	0	0	0	0	0
E	20.8	21.3	20.9	8-33s	80	100	100	100	100	100	0	0	100
				8-46s	33	100	100	100	100	100	0	0	100
				33-31-B	0	0	0	0	0	0	0	0	0

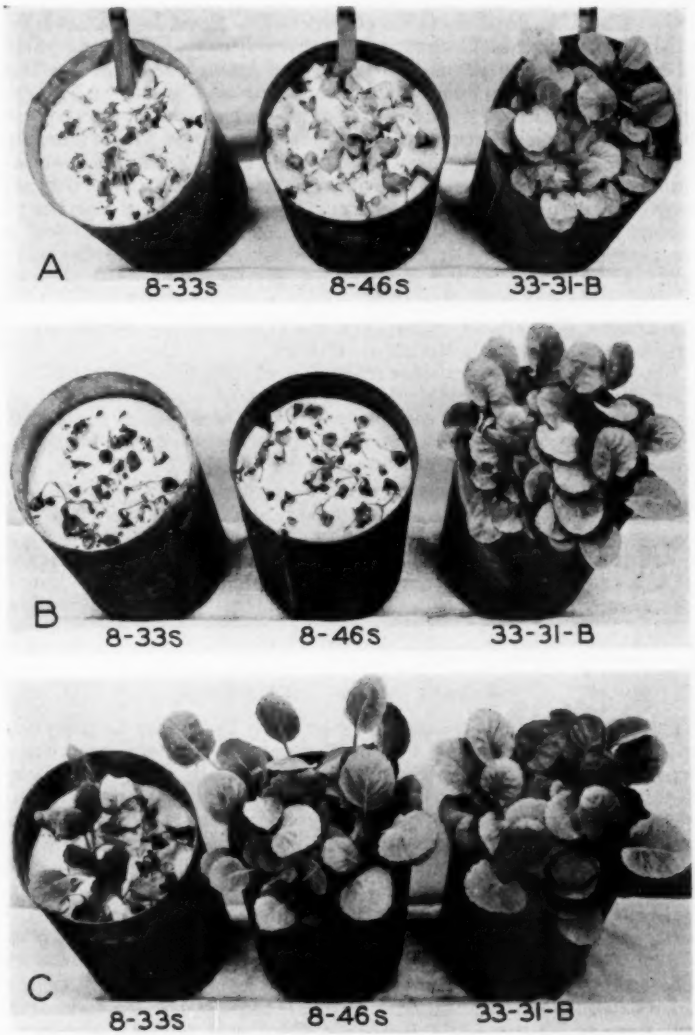

FIGURE 4.—Comparison of two susceptible F_1 progenies, 8-33s and 8-46s, and a homozygous resistant strain, 33-31-B, when grown in inoculated soil at various low soil temperatures. Each can contained 15 plants. A, Plants after 13 days at 20.8° to 21.3° C. (table 7, row E); all plants of 8-33s and 8-46s are diseased; the majority of the latter are still alive. B, Same as A, after 36 days; all plants of 8-33s and 8-46s have succumbed. C, Plants after 36 days at 13.1° to 14.8° (table 7, row C); all plants of 8-33s are diseased and 10 are dead; 7 plants of 8-46s are slightly diseased and the remainder healthy. The homozygous resistant strain, 33-31-B, remained healthy throughout. Note that at 20° there is little difference in reaction between the two susceptible strains, whereas at the lower soil temperature there is considerable difference.

The incubation period of the disease was greatly lengthened at the lower temperatures, from 8 days at 20° to 21° C. to 29 days at 10° to 12°. The time limit must be considered at these low temperatures. In the first experiment 8-46s showed no diseased plants at 13.5° to 13.9° at the end of 23 days (table 6). In the second experiment, however, the disease occurred in this strain at the end of 29 days at 11.8° to 13.4° (table 7).

The critical soil temperature at which incipient yellows occurs in strain 8-33s is probably between 10° and 12° C. These results are quite different from those of earlier workers (*2*, *5*, *6*) who reported the approximate minimum temperature for disease occurrence at 17°. Recently Blank (*1*) has shown that the disease develops at soil temperatures as low as 12° to 13°. His conclusion that the critical soil temperature was near 12° is borne out in the foregoing experiments, and the evidence indicates that the critical soil temperature for the appearance of yellows symptoms may be slightly below 12° for certain very susceptible strains of cabbage.

These experiments indicate that the F_1 progenies from self-pollinated Wisconsin Hollander plants which vary slightly in reaction to the yellows disease have distinctly different degrees of resistance at low soil temperatures. The expression of this resistance is markedly dependent upon the temperature of the soil. For example, strain 8-33s is susceptible to the disease at temperatures as low as 10° to 12° C. At this temperature strain 8-46s remains healthy, but becomes susceptible to the disease at a slightly higher soil temperature. In other words, plants of different progenies differ in genetic composition with reference to number or kind of factors making for resistance. Such a hypothesis would explain the various degrees of resistance found in the different F_1 progenies from self-pollinated Wisconsin Hollander plants.

Relation of Age to Resistance

The idea that the resistance of Wisconsin Hollander to the yellows disease increases with the age of the plants has been accepted for some time. This conception has arisen mainly from the work of Tisdale (*6*), who concluded that older plants are more resistant than younger ones and that resistance becomes more stable as the plants grow older. This conclusion was not borne out in some preliminary tests of the present study and experiments were planned to determine more definitely the relation of age to resistance in this variety.

The first experiment was conducted during November 1930 in a bench one half of which contained inoculated soil and the other half naturally infested soil. The soil temperature was kept quite constant at about 22° C. by means of steam pipes beneath the bench, but varied at times from 19° to 25°. The air temperature was about 22°. Two lots of Wisconsin Hollander (WH-1 and WH-4) and two F_1 progenies from self-pollinated Wisconsin Hollander plants (8-11s and 8-26s) were tested. Seedlings of each strain, previously grown for 30, 45, 60, and 75 days, respectively, on clean soil, were transplanted into each of the two types of infested soil. Because of the diminished sunlight during the early winter months these plants were not as large as plants grown in the spring. The test was continued in the inoculated soil for 20 days and in the naturally infested soil for 25

days. Table 8 shows the percentages of diseased plants at approximately regular intervals. The data indicate that within the limits of the experiment there were no consistent differences in degree of resistance attributable to increase in the age of the seedlings. The rate of occurrence of disease in seedlings of different ages differed but slightly in any single strain. Moreover the older seedlings were as severely diseased as the younger seedlings of the same strain. In relation to one another, the four strains reacted to the disease as in previous soil-temperature tank tests. The incubation period was longer and the disease developed more slowly in the naturally infested soil than in the inoculated soil.

TABLE 8.—*Development of cabbage yellows in two F_1 progenies from self-pollinated Wisconsin Hollander plants and in commercial Wisconsin Hollander seedlings when transplanted at various ages to inoculated or to naturally infested soil and grown at a soil temperature which varied from 19° to 25° C.*

Strain no.	Age of plants when transplanted	Plants transplanted to inoculated soil						Plants transplanted to naturally infested soil					
		Total plants	Plants diseased after—					Total plants	Plants diseased after—				
			7 days	10 days	13 days	17 days	20 days		7 days	10 days	13 days	17 days	25 days
	Days	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
S-11s	30	29	3	24	52	83	86	21	0	5	24	43	67
	45	43	2	21	40	81	84	25	0	4	16	40	72
	60	20	0	10	40	80	80	7	0	14	14	43	71
	75	25	0	32	51	88	88	7	0	14	29	57	86
S-26s	30	25	4	28	80	88	92	18	0	6	17	61	78
	45	20	15	40	85	95	95	9	0	11	33	78	89
	60	30	7	42	63	97	97	11	0	0	27	73	91
	75	25	8	40	64	96	96	9	0	11	22	67	78
WH-1	30	58	10	53	88	98	100	28	4	7	25	71	93
	45	50	12	42	76	92	96	26	0	15	40	73	96
	60	39	8	49	72	97	97	26	0	23	69	88	96
	75	38	13	61	84	95	100	8	0	25	75	100	100
WH-4	30	58	40	90	93	98	100	20	5	25	70	90	100
	45	37	51	84	97	100	100	28	4	43	82	96	100
	60	37	32	73	92	100	100	19	16	37	78	100	100
	75	37	27	78	92	100	100	9	11	56	89	100	100

In the next test seedlings grown from seed in inoculated soil were compared with seedlings that had been grown in clean soil and then transplanted to inoculated soil. This experiment was conducted during March and April 1932 and the tests were made only at the soil temperatures of 20° and 22° C., because, as has been shown, the resistance of Wisconsin Hollander is broken down completely at higher temperatures in inoculated soil. The seeds to be planted in the inoculated soil were first placed on moist filter paper in sterile Petri dishes for 1 day to assure uniform germination. These germinating seeds were planted in inoculated soil simultaneously with seedlings of different ages that had been grown in clean soil. The seedlings were then placed at a rather low temperature (12° to 15°) for 1 day to allow partial recovery from transplanting before they were placed in the soil-temperature tanks. The cans containing the planted seeds were placed immediately in the soil-temperature tanks.

The first symptoms of disease appeared after 7 days at 22° C., and the diseased plants were noted each day thereafter. Table 9 shows

the percentage of diseased plants at various intervals. In all cases, both in this experiment and in duplicate tests, the seedlings grown from seed in inoculated soil were slower in showing symptoms of the disease than were seedlings grown on clean soil for 15 days before being transplanted to inoculated soil. When these results are compared with those of previous experiments, a similar difference in rate of disease occurrence is noted also between seedlings from seed in inoculated soil and 30-day-old transplants. The percentage of diseased plants was markedly smaller in seedlings grown from seed in inoculated soil. This was especially noticeable in the earlier disease counts. Toward the end of the tests the percentage of diseased plants tended to become equal in the seedlings of different ages. The disease appeared earlier and developed more rapidly in the susceptible strain (8-33s) in seedlings of all ages. The relative degrees of resistance of different strains were quite uniform in the seedlings of different ages.

From these results it appears that the resistance of Wisconsin Hollander is an inherent character present in the youngest seedlings, not a character acquired or developed coincidently with the increase in age of the plant. It is possible that vigor and development may modify the expression of resistance in Wisconsin Hollander but this would probably be of minor importance in comparison with the resistance resulting from the genetical constitution of the plant. No such modification was noted in the foregoing tests. These results with young seedlings are quite different from those of Tisdale (*6*), but were consistent in three tests.

TABLE 9.—*Comparison of resistance of Wisconsin Hollander seedlings grown from seed in inoculated soil with that of seedlings grown for various periods in clean soil and then transplanted to inoculated soil*

[Plants were grown at soil temperatures of 20° and 22° C.]

Strain no.	Age of plants when transplanted	Temperature of inoculated soil 20° C.						Temperature of inoculated soil 22° C.					
		Total plants	Plants diseased after—					Total plants	Plants diseased after—				
			8 days	10 days	14 days	16 days	20 days		8 days	10 days	14 days	16 days	20 days
	Days	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
8-33s	[a] 0	42	0	48	93	100	100	30	3	70	97	100	100
	15	20	35	100	100	100	100	20	80	95	100	100	100
	48	12	0	58	100	100	100	15	27	100	100	100	100
	67	12	0	33	100	100	100	12	0	100	100	100	100
8-37s	[a] 0	35	0	9	20	43	66	40	0	13	33	67	78
	15	20	0	35	75	75	90	20	0	35	85	90	90
WH-1	[a] 0							57	0	2	28	77	88
	15	20	0	10	75	80	85	20	0	25	80	90	90

[a] Seed planted in inoculated soil.

F_2 PROGENIES FROM SELECTED WISCONSIN HOLLANDER PLANTS

The reaction of the F_1 progenies from Wisconsin Hollander plants indicates that this variety consists of a heterogeneous group of individuals (table 2), ranging from those highly resistant to those completely susceptible to the yellows disease under field conditions. The

F_1 progenies from the Wisconsin Hollander plants 8-4, 8-11, 8-23, 8-28, 8-39, 8-40, 8-47, and 8-49 were saved in the fall of 1930 for further genetical study. These progenies represented various degrees of resistance exhibited by Wisconsin Hollander plants, and consisted of susceptible crosses, resistant crosses, and progenies from self-pollination.

F_2 PROGENIES FROM SELF-POLLINATION

A series of progenies derived from Wisconsin Hollander plants by two generations of selfing was tested in 1931 in inoculated soil at constant soil temperatures. The first test was carried out in October and November (table 10). At the end of 30 days at a soil temperature of 22° C., many plants of some strains were still apparently healthy. Such individuals were transplanted to a bench containing inoculated soil for an additional test lasting 38 days. The soil temperature in the bench was usually 22°, but varied from 19° to 24°. At the end of this second test, plate cultures were made from all doubtful plants. Eight plants of the strain 8-149s were healthy beyond doubt after a third test at 24°. A few F_2 progenies (8-103s, 8-147s, 8-149s) were more resistant and others were more susceptible than were the parent progenies when tested under similar conditions. (Compare with tables 3 and 4.) Duplicate tests with these strains gave similar results, except that 8-146s and 8-148s showed a small percentage of healthy plants at the end of 30-day tests.

TABLE 10.—*Development of cabbage yellows in F_2 progenies from self-pollinated Wisconsin Hollander plants when grown in inoculated soil at 22° C. for 30 days and then at 19° to 24° for an additional 38 days*

Parent plant no.	Strain no.	Total plants	Plants diseased after—		Parent plant no.	Strain no.	Total plants	Plants diseased after—	
			30 days	68 days				30 days	68 days
		Number	*Percent*	*Percent*			*Number*	*Percent*	*Percent*
8-4s	8-100s	60	85	97	8-39s	8-143s	33	100	100
	8-103s	35	29	74		8-144s	54	100	100
	8-104s	48	54	94		8-146s	36	100	100
	8-109s	19	58	79		8-147s	41	93	93
						8-148s	32	100	100
8-40s	8-136s	73	100	100		8-149s	34	76	79

Although the number of Wisconsin Hollander plants represented by these F_2 progenies is small, the results indicate that resistance to yellows can be increased by selection in self-pollinated lines. The variability of resistance in F_2 progenies suggests the interaction of modifying factors, the reaction of a particular strain depending upon the assortment of modifying factors which it possesses. The high resistance shown by the eight plants from 8-149s is probably the result of a combination of factors very favorable for resistance. It is also possible that these plants resulted from chance contamination or error during pollination.

F_2 PROGENIES FROM SUSCEPTIBLE CROSSES

Twenty-five progenies were obtained from self-pollination of F_1 plants from the cross Wisconsin Hollander × a homozygous susceptible plant. The F_2 progeny tests were conducted in inoculated soil

in Wisconsin soil-temperature tanks, and in a bench containing inoculated soil. The soil temperature of 22° to 23° C. was maintained in the tanks, and in the bench the soil temperature was usually 22° but varied from 19° to 24°. The tests were about 30 days in duration. All progenies showed almost complete susceptibility to yellows, although the Wisconsin Hollander parent plants were of the more resistant classes (table 2). Furthermore, the majority of the F_1 parent plants had been grown on infested soil in 1930, thus representing the survivals of their respective progenies. The same degree of resistance as that of the Wisconsin Hollander parent plants was not recovered in any progeny resulting from selfing plants of the F_1 susceptible cross. Such reactions are not uncommon, however, when the character in question is controlled by modifying factors. These results indicate that the modifying factors which control resistance in Wisconsin Hollander plants are absent in the susceptible line used, and in the resultant cross these factors were dispersed or segregated so that the original resistance was not recovered in the F_2 generation.

F_2 PROGENIES FROM RESISTANT CROSSES

The F_2 progenies obtained by selfing plants from the resistant cross Wisconsin Hollander × 21-30-A were tested in inoculated soil at temperatures of 22° and 22° to 24° C. These tests are summarized in table 11. It is evident that every progeny segregated as a monohybrid, yielding approximately 25 percent of diseased plants. The deviation from the theoretical 3 to 1 ratio is practically negligible. In only one progeny was the deviation three times the probable error, and in this progeny (8-107Js) more than one fourth of the plants were diseased. Among the progenies from crosses with the different Wisconsin Hollander plants there was no apparent difference in respect to rate of disease occurrence or severity of disease in individual plants. The progenies representing the more resistant Wisconsin Hollander plants (8-11, 8-49) gave a closer fit to the 3 to 1 ratio than those representing the more susceptible Wisconsin Hollander plants (8-47, 8-23). This difference is of no significance, however, as all progenies gave a very close fit.

TABLE 11.—*Occurrence of cabbage yellows in the F_2 progenies from self-pollination of the F_1 crosses of Wisconsin Hollander × homozygous resistant plants (table 2) when grown in inoculated soil at constant soil temperatures of 22° to 24° C.*

Wisconsin Hollander parent no.	Progeny no.	Total number of plants		Deviation	Probable error	Dev. / P. E.
		Healthy	Diseased			
8-23	8-99Js	25	8	0.3	1.7	0.2
	8-100Js	138	39	5.3	3.9	1.4
	8-101Js	84	25	2.3	3.1	.7
	8-102Js	38	10	2.0	2.0	1.0
	8-103Js	124	37	3.3	3.7	.9
	8-104Js	52	22	3.5	2.5	1.4
Total		461	141	19.5	7.2	2.7
8-28	8-105Js	83	21	5.0	3.0	1.7
	8-106Js	17	9	2.5	1.5	1.7
	8-107Js	62	34	10.0	2.9	3.5
	8-108Js	54	14	3.0	2.4	1.3
Total		216	78	4.5	5.0	.9

TABLE 11.—*Occurrence of cabbage yellows in the F_2 progenies from self-pollination of the F_1 crosses of Wisconsin Hollander × homozygous resistant plants (table 2) when grown in inoculated soil at constant soil temperatures of 22° to 24° C.*—Con.

Wisconsin Hollander parent no.	Progeny no.	Total number of plants		Deviation	Probable error	Dev. / P.E.
		Healthy	Diseased			
8-39	8-115Js	65	29	5.5	2.8	2.0
	8-116Js	40	13	.3	2.1	.1
	8-117Js	81	25	1.5	3.0	.5
	8-118Js	99	30	2.3	3.3	.7
Total		285	97	1.5	5.7	.3
8-40	8-125Js	31	10	.3	1.9	.2
	8-126Js	108	38	1.5	3.5	.4
	8-127Js	15	7	1.5	1.4	1.1
	8-128Js	85	21	5.5	3.0	1.8
Total		239	76	2.8	5.2	.5
8-47	8-129Js	4	1	.3	.7	.4
	8-130Js	122	32	6.5	3.6	1.8
	8-131Js	93	25	4.5	3.2	1.4
	8-132Js	155	48	2.8	4.2	.7
	8-133Js	70	25	1.3	2.9	.4
	8-134Js	138	43	2.3	3.9	.6
Total		582	174	15.0	8.0	1.9
8-49	8-135Js	77	27	1.0	3.0	.3
	8-136Js	12	7	2.3	1.3	1.8
	8-137Js	65	19	2.0	2.7	.7
	8-138Js	57	17	1.5	2.5	.6
	8-139Js	136	44	.8	3.9	.2
	8-140Js	10	2	1.0	1.0	1.0
Total		357	116	2.0	6.4	.3
8-11	8-144Js	76	20	4.0	2.9	1.4
	8-145Js	71	23	.5	2.8	.2
	8-146Js	31	10	.3	1.9	.2
Total		178	53	4.8	4.4	1.1
Grand total		2,318	735	28.0	16.1	1.7

One fourth of the plants of these F_2 progenies were susceptible at 22° C., showing that the degree of resistance of the Wisconsin Hollander parent plants was not evident under these conditions. It is expected that some plants of the F_2 progenies from susceptible crosses and one fourth of the plants from resistant crosses will exhibit some resistance at lower soil temperatures (18° or 20°) or in the field. Field trials and tests at low soil temperatures with these progenies have not yet been carried out. The foregoing results, however, give further evidence that the resistance exhibited by Wisconsin Hollander is genetically different from and independent of that shown by the homozygous resistant lines. They further indicate that this resistance is a result of modifying factors.

DISCUSSION

The object of the investigation reported herein was to secure more exact information regarding the nature of resistance in Wisconsin Hollander to the yellows disease of cabbage. The evidence from this study and from observations of earlier workers indicates that with regard to resistance the Wisconsin Hollander variety consists of a heterogeneous group of individuals. This is shown by the F_1 progenies

from self-pollinated Wisconsin Hollander plants, which, when grown in the field, differed widely in their reaction to the disease, some being very susceptible, some highly resistant, and some of intermediate degrees of resistance.

The studies with the progenies from self-pollinated Wisconsin Hollander plants and from crosses with homozygous resistant and homozygous susceptible plants of other varieties indicate that the resistance displayed by Wisconsin Hollander is controlled by modifying factors. These factors of resistance are different in type and reaction from the single dominant factor controlling resistance in the homozygous resistant lines. The factors for resistance in Wisconsin Hollander are not dominant but are cumulative in effect. It is suggested that the two mother plants from which the Wisconsin Hollander variety was developed probably contained a considerable number of modifying factors. As a result of segregation through successive generations, plants of this variety would be heterogeneous in nature, differing from one another in the number and kind of the factors that produce resistance. Some of the plants, when grown under field conditions, would be very susceptible, some highly resistant, and many of intermediate degrees of resistance. This was shown to be true in field tests with progenies from self-pollinated Wisconsin Hollander plants (table 2), and partly accounts for the differences among commercial lots of Wisconsin Hollander. If propagation occurs for a period of years on soil free from the yellows organism, where elimination of susceptible plants is impossible, the percentage of susceptible plants may increase.

Genetical studies with Wisconsin Hollander × homozygous resistant and Wisconsin Hollander × homozygous susceptible crosses indicate that the modifying factors for resistance in Wisconsin Hollander are lacking in the two respective homozygous strains. The F_1 progenies from the susceptible crosses were very susceptible in the field tests. The relative degrees of resistance of the Wisconsin Hollander parents were partially expressed in these tests in that progenies from crosses with the more resistant Wisconsin Hollander parents showed a smaller percentage of diseased plants. These progenies were completely susceptible when grown in inoculated soil at a constant soil temperature of 22° C. The F_2 progenies from susceptible crosses were also very susceptible when grown in inoculated soil at a constant soil temperature of 22°. Assuming the absence of modifying factors for resistance in the homozygous susceptible plants, the F_1 progenies from crosses with Wisconsin Hollander would segregate for the factors of the Wisconsin Hollander parent, and therefore the degree of resistance would be decreased. The original resistance of the Wisconsin Hollander parents was not recovered in the F_2 generation. Total recovery of this resistance is expected in but few plants in successive generations if the number of factors is large. It is expected that some resistance will be shown by F_2 plants from the foregoing crosses if they are grown under lower soil-temperature conditions or in the field. Additional tests in this respect were impossible because of the limited seed supply. The F_2 progenies obtained by selfing plants of the resistant cross showed one fourth of the plants susceptible to yellows when grown in inoculated soil at 22° C. The only type of resistance obtained in these tests resulted from the

single dominant factor for resistance of the homozygous resistant parent.

Greater uniformity in resistance to the yellows disease was found in the individuals of F_1 progenies from self-pollinated Wisconsin Hollander plants than was found in Wisconsin Hollander plants in general. Segregation took place in these progenies, however, resulting in some individuals being more resistant and some less resistant than the mean. This was further shown in tests with F_2 progenies from self-pollinated Wisconsin Hollander plants (table 10), some of which were more resistant and others more susceptible than the corresponding F_1 progenies. There was also some difference in resistance among individuals of the F_2 progenies, showing that the character of resistance had not become stabilized by self-pollination in a period of two generations.

The resistance of Wisconsin Hollander as compared with that of homozygous resistant plants is of an intermediate type. The most resistant line from Wisconsin Hollander plants showed complete breakdown of resistance when grown in inoculated soil at a constant soil temperature of 24° C., and but a small percentage of plants remained healthy at 22°; whereas the plants of the homozygous resistant strains remained completely healthy in inoculated soil at temperatures as high as 26°. The resistance of Wisconsin Hollander can be increased by selection in self-pollinated lines, but whether a resistance equal to that of homozygous resistant lines can be developed in Wisconsin Hollander as a result of the accumulation of modifying factors remains to be determined by further study. Such a study should proceed by continued selection of the most resistant plants and self-pollination for two or more generations, until segregation ceases and a high degree of resistance becomes stabilized.

There appears to be a fine balance between the resistance of Wisconsin Hollander plants of the same genetical constitution and the aggressiveness of the pathogene in causing disease. By varying environmental conditions, such as soil temperature, this balance may be easily shifted to favor either the host or the pathogene. Such balances of slightly different planes are assumed for the F_1 progenies of slightly different degrees of resistance to yellows. The shifting of this balance in favor of the pathogene has been noted in various tests. Several F_1 progenies from self-pollination tested in the field in 1930 (table 2, class 3) contained very few diseased plants, and these showed but incipient symptoms. In the warmer summer of 1931, however, 50 percent or more of the plants of these progenies were diseased (table 3), and the diseased plants were more severely affected in general. This partial breakdown of resistance is even more evident on comparing the results of the two field tests of the F_1 progenies from susceptible crosses. Because of the higher degree of resistance found in strain 8-11s, the host-parasite relationship in this strain was but slightly affected by the increase in severity of weather conditions.

This relationship was also noticed in the greenhouse tests. There appears to be a quite definite limit within which the plants of a progeny are successful in resisting the fungus. This limit differs with the different progenies from self-pollinated Wisconsin Hollander plants, which showed various degrees of resistance. For example, very slight symptoms were found in a few plants of strain 8-11s at 16° C.,

of strain 8-46s at 13° to 14°, and of strain 8-33s at 10° to 12°. At these temperatures the majority of plants remained healthy and plants showing symptoms were not retarded in growth but tended to recover. In each case, at slightly higher temperatures, the percentage of affected plants increased and the disease became more severe.

When plants were grown in inoculated soil at 22° C. the majority of those of the most resistant line from selected Wisconsin Hollander (8-11s) showed symptoms of the disease. Many such plants exhibited but slight symptoms and later recovered.

SUMMARY

This paper reports a study of the nature of resistance of the Wisconsin Hollander variety of cabbage to the yellows organism, *Fusarium conglutinons* Wr. A number of plants of this variety were self-pollinated and crossed with homozygous resistant and homozygous susceptible plants from other varieties. Selected plants from the F_1 progenies were self-pollinated.

Field and greenhouse trials were conducted in which the various progenies and commercial Wisconsin Hollander were tested for resistance to yellows in comparison with homozygous resistant and homozygous susceptible lines.

The Wisconsin Hollander variety contains a heterogeneous group of individuals. Under severe field conditions the individuals of F_1 progenies from self-pollinated Wisconsin Hollander plants ranged in degree of resistance from completely susceptible to highly resistant, indicating the existence of modifying factors for resistance. The original resistance of Wisconsin Hollander was not recovered in F_2 progenies from crosses with homozygous susceptible plants. In the F_2 progenies from the cross Wisconsin Hollander × homozygous resistant, when grown in inoculated soil at a constant soil temperature of 22° C., segregation resulted in 3 resistant to 1 susceptible.

Segregation of factors for resistance occurred in the F_1 and F_2 progenies from self-pollinated Wisconsin Hollander plants. Some F_2 progenies exhibited a higher degree of resistance and others a lower degree than did the progenies from which the mother plants were selected.

Individual plants of the more resistant progenies from Wisconsin Hollander are less severely affected by the disease than those of the susceptible progenies. Many plants of the former exhibit but slight symptoms in the field or at a controlled soil temperature of 20° to 22° C. The growth of such plants is not materially checked by the disease and they tend to recover

The resistance of Wisconsin Hollander tends to be broken down when plants are grown in inoculated soil at constant soil temperatures ranging from 20° to 24° C. The most resistant line obtained from Wisconsin Hollander plants became 100 percent diseased at a constant soil temperature of 24°. In comparison with the resistance displayed by homozygous lines the resistance of Wisconsin Hollander is of an intermediate degree.

Wisconsin Hollander seedlings of various ages reacted quite similarly to the disease. There was no evidence of increased resistance

with age in seedlings within the limits studied. The rate of incidence of the disease was slower in seedlings grown from seed in inoculated soil than in 15-day-old plants transplanted to inoculated soil.

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INHERITANCE OF SEED COLOR IN CROSSES OF BROWN-SEEDED AND WHITE-SEEDED SORGHUMS[1]

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INTRODUCTION

In the improvement of sorghums by crossing, the problem of combining desirable characters of brown-seeded varieties with the white-seed character is of both practical and theoretical importance. Stock and poultry feeders generally recognize the greater palatability of white-seeded grain sorghums, and the market value of the better brown-seeded varieties is less than that of other grain sorghums.

Swanson [2] indicated the possible color factorial constitution of a number of brown-, white-, yellow-, and red-seeded sorghums. His conclusions regarding the color factors of the brown-seeded varieties, with the exception of Red Amber, which he determined by genetic experiments, were based upon a microscopic examination of the seed coats.

Data on inheritance of seed color in three crosses between brown-seeded and white-seeded varieties are presented in this paper.

CROSSES STUDIED

The crosses were made by the writer at the Southern Great Plains Field Station, Woodward, Okla., in 1928, 1929, and 1930. The F_2 or segregating generations were grown and observed in 1930, 1931, and 1932. The three crosses were (1) Scarborough broomcorn (C.I.[3] no. 817) × a white-seeded fixed segregate of Freed sorgo × Evergreen Dwarf broomcorn, (2) Blackhull kafir (C.I. no. 813) × darso, and (3) Blackhull kafir (C.I. no. 813) × Sumac sorgo (F.C.I.[4] no. 1712).

Cross no. 1 was made primarily to obtain a white-seeded variety of broomcorn. Scarborough broomcorn has the brown seeds typical of broomcorn. The other parent of this cross, a selection from a cross between Freed sorgo and Evergreen Dwarf broomcorn, was early in maturity and had white seeds and a broomcornlike head but with an inferior brush.

Cross no. 2 was made for the purpose of obtaining a white-seeded strain of darso. The kafir parent, known locally as Sharon or Santa Fé kafir, is white seeded and of medium early maturity. In common with other typical kafirs it has awnless glumes. Darso, the pollen parent, has reddish-brown seeds and is awned.

Cross no. 3 was made to obtain a white-seeded strain of Sumac sorgo. Sumac sorgo, the staminate parent, has very small, dark walnut-brown seeds. The other parent was the kafir used in cross no. 2. The feathery stigmas of the two parents differ in color, those of the kafir being white and those of Sumac sorgo a deep yellow.

[1] Received for publication July 3, 1933; issued November 1933.

[2] SWANSON, A. F. SEED-COAT STRUCTURE AND INHERITANCE OF SEED COLOR IN SORGHUMS. Jour. Agr. Research 37: 577-588. illus. 1928.

[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

[4] F.C.I. refers to accession number of the Division of Forage Crops and Diseases.

The seed-color factors in these several crosses are designated [5] *B*, a factor for brown nucellar layer which also causes a brown in the epidermis; *b*, its allelomorph for absence of the brown nucellar layer; *R*, a dominant factor for red or yellow in the outer layers of the pericarp; and *r*, its allelomorph for lack of red or yellow in this region.

The seed colors of red kafirs and yellow milos appear different to the eye. The red and yellow seed characters have been found to differ from those of white seeds by single dominant factors. In a cross of Early Red kafir and Dwarf Yellow milo made in 1927 the F_2 population of 661 plants produced in 1929 did not contain any white-seeded plants. If the red and yellow color factors had been genetically distinct and independent, one sixteenth of the population, or about 41 plants, should have had white seeds. There were various shades of red, orange, and yellow, but these variations probably were due to genetic or physiologic modifiers, including differences in texture or thickness of the various layers of the pericarp and endosperm. This behavior indicates that the color in the seeds of red kafirs and yellow milos is due to the same single-color factor designated *R*.

EXPERIMENTAL RESULTS

SEED COLOR

In 1930 four 8-rod rows of the F_2 generation of the cross between Scarborough broomcorn and the white-seeded segregate of Freed sorgo × Evergreen Dwarf broomcorn were grown. Six 8-rod rows of the F_2 generation cross of Blackhull kafir × darso were grown in 1931. Three 8-rod rows of the F_2 generation cross of Blackhull kafir × Sumac sorgo were grown in 1932. Seed-color counts were made in each of these crosses. The data are shown in table 1.

TABLE 1.—*Seed color in the F_2 generation of 3 crosses between brown- and white-seeded sorghums*

Cross and items of comparison	Number of plants				χ^2	P
	Total	Nucellar layer present	Nucellar layer absent			
		Brown seeds	Red or yellow seeds	White seeds		
Scarborough broomcorn × white-seeded segregate of Freed sorgo × Evergreen Dwarf broomcorn:						
Observed	733	552	144	37		
Calculated (12:3:1)	733	549.8	137.4	45.8	2.017	0.366
Blackhull kafir × darso:						
Observed	1,367	1,020	269	78		
Calculated (12:3:1)	1,367	1,025.3	256.3	85.4	1.30	.535
Blackhull kafir × Sumac sorgo:						
Observed	684	507	141	36		
Calculated (12:3:1)	684	513	128.3	42.7	2.38	.313

There was a segregation closely approximating a dihybrid 12:3:1 ratio for brown-, yellow-, and white-seeded plants in each of the three crosses.

[5] SIEGLINGER, J. B. SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES. Jour. Agr. Research 27: 53-64. 1924.

White-seeded segregates in the broomcorn cross were selected in F_2, but the inheritance of seed color was not studied in later generations, except to note that the white segregates, when selfed, produced only white-seeded progeny, as expected. The F_3 generation of the Sumac sorgo cross has not yet been grown. The progeny of 50 selected F_2 heads of the Blackhull kafir × darso cross, of which 25 had been selfed, was grown in the F_3 generation in 1932. The breeding behavior of these strains is shown in table 2.

TABLE 2.—*Breeding behavior in F_3 of the progenies from 50 selected F_2 heads of the Blackhull kafir × darso cross, 1932*

Item	Number in F_3 generation				Deviation from 3:1 ratio	Probable error of deviation	Suggested genotype
	Strains	Plants with—					
		Brown or buff seeds	Yellow seeds	White seeds			
Brown seed	17						
Bred true—dark brown	3	All					*BBRR*
Bred true—buff	2	All					*BBrr*
Segregated—brown and buff	4	All					*BBRr*
Segregated—brown and yellow	1	184	53		6.25	4.50	*BbRR*
Segregated—brown (buff) and white	3	546		194	9.0	7.9	*Bbrr*
Segregated—brown, yellow, and white	4	729	190	72	(a)		*BbRr*
Yellow seed	10						
Bred true—yellow	3		All				*bbRR*
Segregated—yellow and white	7		1,276	440	11	12.1	*bbRr*
White seed	23						
Bred true—white	23			[b] All			*bbrr*

[a] $P=0.37$ on basis of calculated 12:3:1 ratio.
[b] Except a few outcrosses from unbagged heads.

The results obtained in the F_3 generation substantiate the 2-factor hypothesis indicated by the F_2 segregation in the three crosses. It seems evident that the brown-seeded varieties, Scarborough broomcorn, darso, and Sumac sorgo, have the factorial constitution *BBRR*, and that the white-seeded varieties have the double recessive factors *bbrr*. The F_1 brown-seeded plants have the factors *BbRr*. The nine possible genotypes, all of which were demonstrated by the F_3 generation behavior of the Blackhull kafir × darso cross, are shown in table 2.

AWNS

A study of the inheritance of awns was made in the Blackhull kafir × darso cross. The kafir parent is awnless, while darso has well-developed straight awns averaging about one half inch in length. The F_1 glumes were awnless like those of the kafir parent, but in the F_2 generation there was a segregation into awned and awnless types. The segregations for awn type and seed color are shown in table 3.

A total of 1,052 plants were awnless and 315 were awned, a deviation of 26.75 ± 10.79 from a calculated 3:1 ratio that probably is not significant. The data in table 3 show a good fit for independent segregation of the factors for awn type and seed color and indicate an absence of linkage.

TABLE 3.—*Awn type and seed color, in the F_2 generation, of the 1,367 plants of the Blackhull kafir×darso cross, 1931*

Awn type and seed color	Number of plants		Awn type and seed color	Number of plants	
	Observed	Calculated (36:12:9:3:3:1)		Observed	Calculated (36:12:9:3:3:1)
Awnless, brown	788	768.9	Awnless, white	58	64.1
Awned, brown	232	256.3	Awned white	20	21.4
Awnless, yellow	206	192.2			
Awned, yellow	63	64.1	Total	1,367	(a)

[a] $\chi^2=4.46$; $P=0.488$.

In the F_3 generation (table 4) all awned plants were obtained from the 14 awned F_2 heads. The 36 awnless heads produced only 5 progenies that were all awnless; the remaining 31 progenies were heterozygous for awns and segregated into a total of 5,962 awnless and 1,800 awned plants, a deviation of 140.5 ± 25.73 from a 3:1 ratio. With random sampling, 36 awnless F_2 heads should produce 12 all-awnless rows, whereas only 5 were produced. The heads used for seed were selected for size of head and seed, small glumes, and the shape of head approximating that of darso. This selection may account for the excess of heterozygous over homozygous awnless heads. The considerable excess of awnless heads in the segregating progenies may have resulted partly from natural crossing in the open-pollinated heads. The segregation in the selfed F_3 lines (table 4) as well as in the F_2 generation (table 3) was in close agreement with the expected numbers from a simple 3:1 ratio.

TABLE 4.—*Awnedness in F_3 of the progenies from 50 selected F_2 heads of the Blackhull kafir×darso cross in 1932*

Awn character in F_2 and breeding behavior in F_3	Number of strains		Number of F_3 plants			Probable error of deviation
	Total	Selfed	Awnless	Awned	Deviation from 3:1 ratio	
Awnless:						
Bred true—awnless	5	2	[a]All			
Segregated (selfed lines)	14	14	2,615	848	17.75	17.19
Segregated (open-pollinated)	17	0	3,347	952	122.75	19.20
Total	31	14	5,962	1,800	140.5	25.73
Awned:						
Bred true—awned	14	9		(a)		

[a] Except a few outcrosses from unbagged heads.

STIGMA COLOR

To study the relation, if any, between stigma color and seed color, the plants of the F_2 generation of the Blackhull kafir×Sumac sorgo cross were tagged according to stigma color during the blooming period. Later, in classifying the seed color, the stigma color was noted from the tags. The following results were obtained:

All the 36 white-seeded plants had white stigmas.
All the 141 yellow-seeded plants had yellow stigmas.
One hundred and ten of the brown-seeded plants had white stigmas.
Three hundred and ninety-seven of the brown-seeded plants had yellow stigmas.

The brown seeds from flowers with white stigmas were lighter in color than those from flowers with yellow stigmas and could best be described as tan or buff like the varieties and segregates having the *Br* factors. Judged from the stigma color of the white- and yellow-seeded segregates, it may be concluded that in this cross the yellow stigma color is merely another expression of the presence of the *R* color factor and that the white stigma indicates an absence of the *R* factor, or that complete linkage existed between the *R* factor and the colored stigma. This knowledge should make it possible to identify one fourth of the white-seeded types at the beginning of the blooming period and thus eliminate the bagging in the three fourths of an F_2 population having the *R* factor for seed color when white-seeded segregates only are desired.

SUMMARY

Data on the inheritance of the seed color of three crosses between brown-seeded and white-seeded sorghums are presented.

The brown seed color of Scarborough broomcorn, darso, and Sumac sorgo is the result of two seed-color factors, *B* and *R*.

In the F_2 generation of crosses between the brown-seeded and white-seeded sorghums, a ratio of 12 brown- to 3 yellow- to 1 white-seeded plant was obtained.

The awnless condition in Blackhull kafir is dominant to the presence of awns in darso, and a segregation into 3 awnless to 1 awned was observed in the F_2 generation of this cross. There was no apparent linkage of the seed color and awn factors.

The stigma color and its relation to seed color was studied in the F_2 generation cross of Blackhull kafir×Sumac sorgo. Yellow stigma was completely associated with the presence of the *R* seed-color factor.

SUBERIZATION AND WOUND-CORK FORMATION IN THE SUGAR BEET AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY[1]

By ERNST ARTSCHWAGER, *pathologist*, and RUTH COLVIN STARRETT, *assistant cytologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

A large percentage of sugar beets are stored at harvest before passing through the factory, and, according to Tompkins and Nuckols,[2] in such storage piles crown rot frequently develops. These writers found the resulting injuries most pronounced in beets topped at or below the line of the lowest leaf scar and only slight where in topping a large part of the crown was allowed to remain. Unable to account for this differential decay, they suggest that a study of wound-cork formation might aid in the solution of the problem.

While plant organs, such as tubers, fleshy roots, and corms, normally respond to wounding by rapid suberization of the cut surface and a subsequent development of a firm cork layer, Priestley and Swingle[3] have found that beets form a visible suberin deposit only slowly.

The studies reported in this paper were initiated to determine the influence of temperature and humidity on the rate of suberization and wound-cork formation on beets topped at different planes, the method of procedure being essentially the same as that employed in the study of wound-periderm development in sweetpotato and gladiolus.[4]

STRUCTURE OF THE MATURE BEET

The sugar beet (fig. 1) is an elongated, more or less pear-shaped body composed morphologically of crown, neck, and root. In cross section it shows a small, star-shaped core surrounded by a number of annular zones or rings of growth. Each annular zone is made up of a concentric ring of vascular tissue and storage parenchyma. The vascular tissue is composed of numerous collateral bundles which anastomose tangentially, forming a network of connecting bundles. In the narrow tapering zone of the beet the rings unite with one another gradually, but a more abrupt fusion is effected in the crown. This zone is composed essentially of intricately anastomosing vascular bundles mostly devoid of the parenchymatous elements so characteristic of the main body of the beet. In the upper region of the neck the central core opens up and encloses pith, which, as the neck passes into the crown, widens and frequently becomes hollow.

[1] Received for publication June 2, 1933; issued November 1933.

[2] TOMPKINS, C. M., and NUCKOLS, S. B. THE RELATION OF TYPE OF TOPPING TO STORAGE LOSSES IN SUGAR BEETS. Phytopathology 20:621-635, illus. 1930.

[3] PRIESTLEY, J. H., and SWINGLE, C. F. VEGETATIVE PROPAGATION FROM THE STANDPOINT OF PLANT ANATOMY. U.S. Dept. Agr. Tech. Bul. 151, 99 p., illus. 1929.

[4] ARTSCHWAGER, E., and STARRETT, R. C. SUBERIZATION AND WOUND-PERIDERM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY. Jour. Agr. Research 43:353-364, illus. 1931.

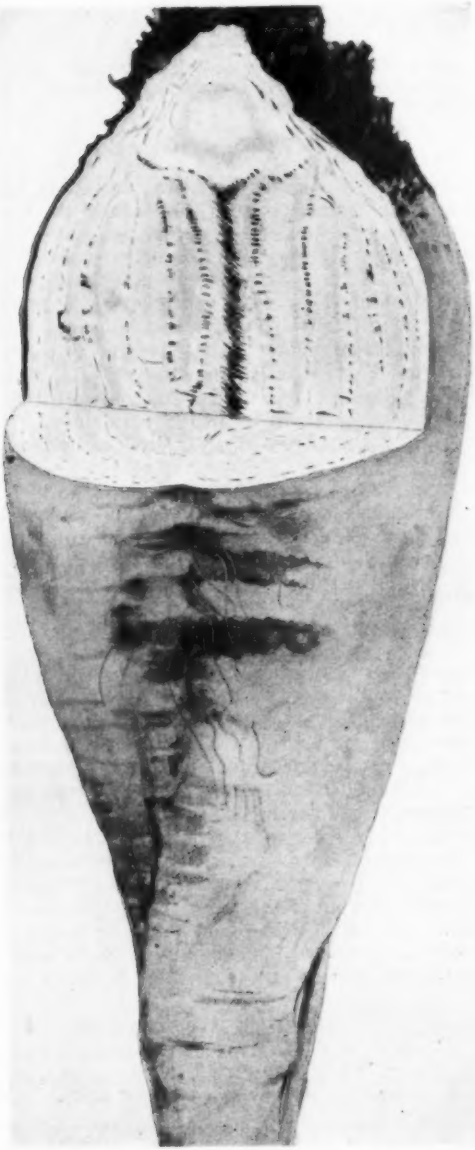

FIGURE 1.—Sugar beet cut to show transverse and tangential surfaces.

SUBERIZATION AND WOUND-CORK FORMATION

The rate of suberization and wound-cork formation as determined from a series of samples taken from an experiment in which temperatures and humidities were closely controlled is given in figure 2, the lightly shaded area indicating surface suberization and the heavily shaded area deeper suberin deposits. The latter, however, even under optimum conditions is slow in forming, at best involving only one or two cell layers. The contour of the suberized zone appears to be related to the tissues exposed in the cut. In the narrow, cortical region the suberin layer is sunk more or less deeply into the tissue beneath the exposed surface, while in other regions the topography of this layer is practically flat.

At low temperatures suberization of the cut surface is both slow and shallow; at higher temperatures its development is initiated after the third day and maximum development is attained after 5 to 7 days. Changes in relative humidity seem to have little influence on surface suberization, except that at maximum storage temperature the humidities below saturation seem to stimulate an early development of deeper-reaching suberin deposits. This condition is contrary to observations made on sweetpotato and gladiolus, which reacted favorably to increases in the relative humidity.

Since suberization precedes wound-periderm formation, the factors governing the former process will also affect the latter. However, while somewhat lowered humidities expedited suberization, a wound periderm would only develop when both temperature and relative humidity were high. The periderm layer, however, was very shallow (fig. 3), even under optimum conditions for its development. The first periderm cells were observed in the cortex of the cut surface, and this layer also attained its maximum thickness at this point. This ready response of cortical cells to wounding is even more pronounced in sweetpotato and gladiolus than in beet, and very likely it is a general phenomenon.

A comparative study of wound healing in crown, neck, and tap region of beets showed little difference in either manner or rapidity of suberization and wound-cork formation. In beets topped through the upper crown suberization was somewhat more rapid, but a wound periderm was slow in forming and could not have been a factor in preventing early infection in storage piles. The fact remains, however, that crown topping results in a considerable decrease in the percentage of decay in beets in storage piles as compared with that in beets topped at or below the lowest leaf scar; and since difference in suberization rate in beets cut through the crown or below the leaf scars is rather slight, the peculiar anatomical structure of the crown may in itself constitute an effective barrier to pathogenes. Here the vascular bundles anastomose freely and older bundles run obliquely, almost horizontally from the core to the periphery. There is little intervening parenchyma but a great deal of phloem tissue, and because the bundles run mostly horizontally there are also fewer exposed bundle ends. Phloem is known to actively stimulate suberin deposits, and its preponderance in the crown region may possibly cause the early formation of a slight yet effective suberin deposit, although the latter may not be detectable with the common reagents.

In the selection of mother beets for seed production, borings are commonly made for the removal of pulp for sugar analysis (fig. 4).

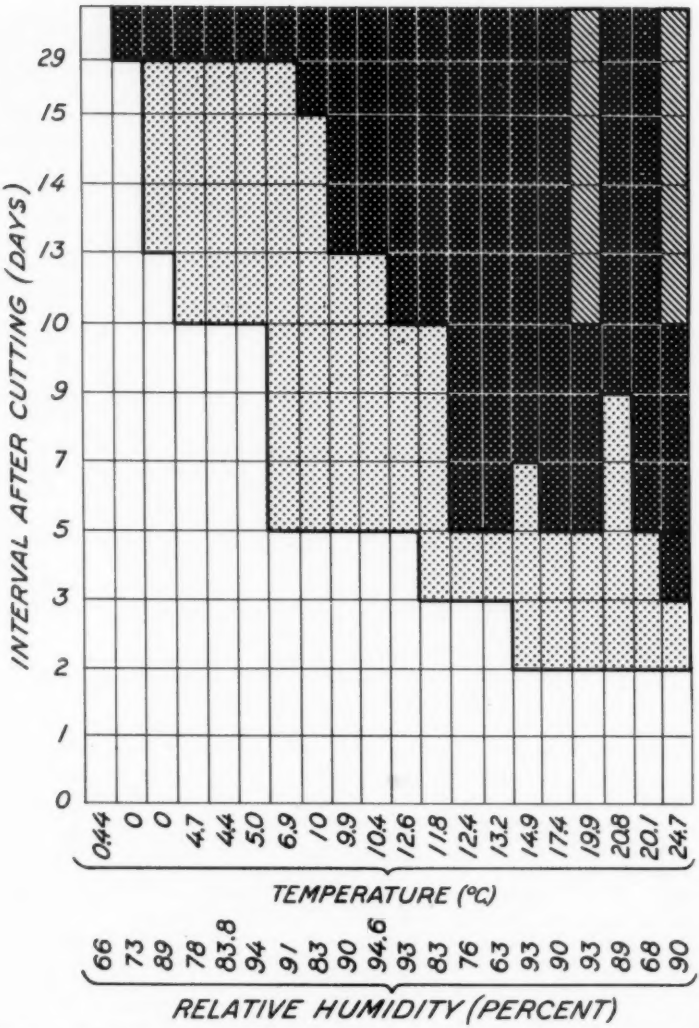

FIGURE 2.—Suberization and wound-periderm formation in sugar beet. Light shading indicates surface suberization; heavy shading, deeper suberin deposits; and hatching, wound periderm.

The outer surface of such a bore from beets that had been kept at a low storage temperature for a number of months was dark and when cut with a knife proved to be hard and gritty. Its outer zone, several cells deep, was lignified, and only one cell layer between lignified and

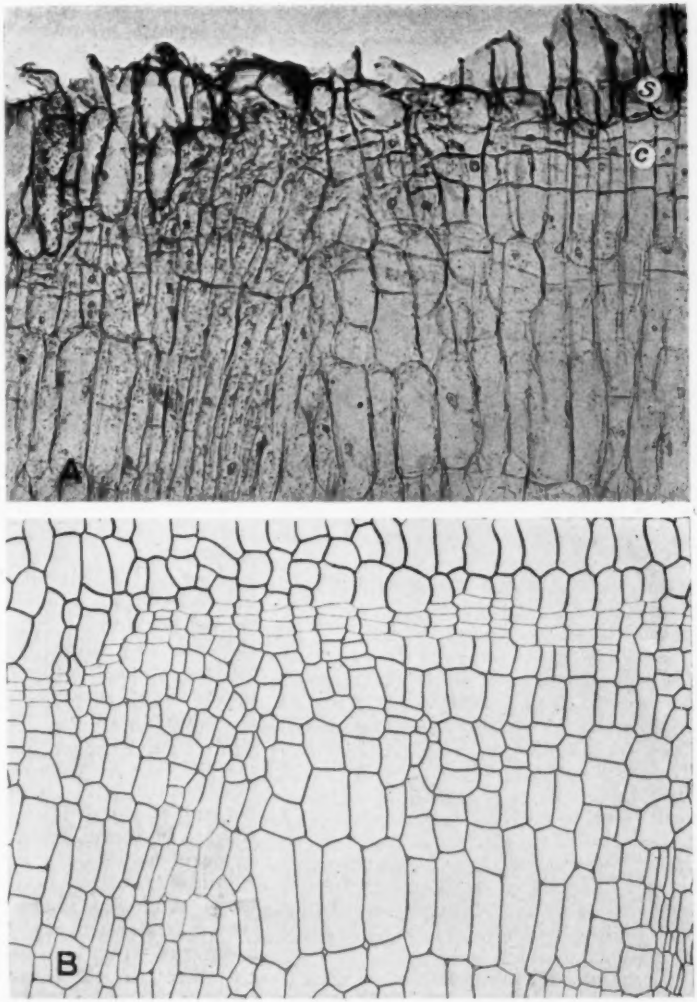

FIGURE 3.—*A*, Wound-periderm formation in sugar beet kept for 10 days at a temperature of 19.9° C. and a relative humidity of 93 percent; *s*, suberized; *c*, wound cork; *B*, detailed drawing of *A*.

normal tissue took the purple ammoniacal gentian-violet stain. There was local evidence of a wound periderm, but this tissue never formed an uninterrupted concentric band, and some material showed no

evidence whatsoever of wound-periderm formation. Although no observations were made on the time factor in suberization, it was evident that the lignified and suberized zone bordering the bore hole afforded considerable protection against infection.

FIGURE 4.—Longitudinal section of sugar beet with bore hole.

The much-belated appearance of wound cork in this material indicates that even at low storage temperatures a periderm may eventually develop, although no periderm formation was apparent in the course of an experiment that ran for 30 days. This observation on mother beets is comparable with the results obtained with gladiolus corms, in which at low temperature a periderm developed only after 79 days.[5]

SUMMARY

The roots of sugar beet respond to wounding by suberization of the cut surface and a belated development of a shallow wound periderm. Suberization is very slight; it involves, at best, only one or two layers of cells, and if temperature and relative humidity are not favorable it is found only in the outer wall. Under favorable conditions, a wound periderm develops as early as 10 days after wounding, but this layer is very shallow. There is little difference in the healing of wounds in beets cut through the crown, neck, or tap region, and the relative scarcity of decay in beets topped through the crown is probably bound up with the specialized anatomical structure of this region. The tissues bordering bore holes made through beets become lignified and slightly suberized. In addition there is slight localized periderm formation.

[5] ARTSCHWAGER, E., and STARRETT, R. C. (See footnote 4.)

LEPTOSPHAERIA SALVINII, THE ASCIGEROUS STAGE OF HELMINTHOSPORIUM SIGMOIDEUM AND SCLEROTIUM ORYZAE[1]

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INTRODUCTION

Stem rot of rice (*Oryza sativa* L.), caused by *Sclerotium oryzae* Catt., has long been known to cause severe losses to rice growers both in the United States and in foreign countries. The fungus was first described from Italy in 1876 by Cattaneo (*3*).[2] In 1907 Metcalf (*8*) reported that it had been found in North Carolina and that it "caused a stem rot under water."

In 1921 Tisdale (*18*) reported the occurrence of the disease in Louisiana and stated that he suspected its occurrence in Arkansas. He also demonstrated the pathogenicity of the fungus by adequate inoculation experiments. In 1926 Young (*22*) definitely reported the disease in Arkansas.

In 1910 Miyake (*9*) reported the occurrence of stem rot in Japan. He stated that great losses were caused by this disease because of the failure of the rice grains to fill properly. Subsequent investigations were made by Sakurai (*14*), Hemmi and Yokogi (*7*), and others. In Cochinchine the disease was reported by Vincens (*21*) in 1923. In 1913 Shaw (*15*) and Butler (*2*) stated that stem rot occurred in India and that one of the most conspicuous symptoms was the stimulation of tillering, a symptom that has not been observed in the United States. Additional reports of stem rot have been made from various parts of India by Bryce (*1*), Thompstone and Sawyer (*17*), Rhind (*13*), and Park (*10*); from the Philippines, by Teodoro and Bogayong (*16*) and Reyes (*11*, *12*); and from Bulgaria by Dodoff and Kovachevsky (*6*).

All the investigators mentioned except Vincens give only the sterile fungus, *Sclerotium oryzae*, as the cause of stem rot. Vincens (*21*), on his return to France from Cochinchine, carried on a series of experiments with *S. oryzae* and reported the development of a number of conidial forms associated with it in culture. These were *Verticillium* sp., *Beauveria oryzae* Vincens, forms alpha and beta; *Fusarium oryzae* Vincens, forms alpha and beta; and *Acremonium fuliginosum* Vincens. Descriptions and illustrations of these were given, but it was not shown that any of them were connected with the stem-rot fungus, *S. oryzae*.

The writer (*19*, *20*) has previously reported briefly the results of investigations showing that the stem-rot fungus (*Sclerotium oryzae* Catt.), has a conidial stage (*Helminthosporium sigmoideum* Cav.)

[1] Received for publication June 27, 1933; issued November 1933. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and the Arkansas, Louisiana, and Texas Agricultural Experiment Stations.

[2] Reference is made by number (italic) to Literature Cited, p. 685.

and also an ascigerous stage (*Leptosphaeria salvinii* Catt.). The present paper reports these results in full.

HELMINTHOSPORIUM SIGMOIDEUM, THE CONIDIAL STAGE OF SCLEROTIUM ORYZAE

In the summer of 1930 the writer found conidia and conidio hores of a *Helminthosporium* on agar cultures of *Sclerotium oryzae* and also on some seedlings of rice grown under aseptic conditions in test tubes and inoculated with a pure culture of *S. oryzae*. Inoculations with sclerotia of *S. oryzae* on other sterile rice seedlings gave the same results. Transfers made at the same time from the original culture failed to show the presence of any contaminating organism. More extensive inoculations and a detailed study of the conidial stage were then made. In the fall of 1931, 16 commercial varieties of rice grown at the Rice Branch Experiment Station, Stuttgart, Ark., were examined for this *Helminthosporium*. Spores and conidiophores of the fungus were found on mature stubble of all the varieties.

SOURCE OF CONIDIA

Sterile rice seedlings grown on agar in test tubes were inoculated with cultures of *Sclerotium oryzae* from the United States, Japan, and India, respectively. In these experiments eight cultures of *S. oryzae* from the following sources were used:

Culture no. 1 isolated by V. H. Young, Department of Plant Pathology, University of Arkansas, from diseased rice collected by him in the vicinity of Stuttgart, Ark.

Culture no. 2 isolated by the writer from diseased rice collected by him in the vicinity of Beaumont, Tex.

Culture no. 3 received from the Centraal Bureau voor Schimmelcultures, Baarn, Netherlands. This culture was isolated from diseased rice in Japan by K. Nakata, Kyushu Imperial University, Fukuoka, Kyushu, Japan.

Culture no. 4 obtained from India through the courtesy of M. Mitra, imperial mycologist, Imperial Institute of Agricultural Research, Pusa, India.

Cultures nos. 5 to 8 obtained from India through the courtesy of B. B. Mundkur, of the Imperial Institute of Agricultural Research, Pusa, India.

In all the inoculations in test tubes the sclerotia were placed on the agar at the side of the seed after the seed had germinated and the seedling had been found to be sterile. Following inoculation, the seedlings were incubated at about 25° C. The seedlings were not wounded.

On January 7, 1930, culture no. 1 was used in making inoculations on five sterile Blue Rose rice seedlings grown singly in test tubes on corn-meal agar. Fifteen days later conidia of a *Helminthosporium* had been produced on four of the seedlings. By that time all the inoculated seedlings had succumbed to the attacks of the fungus, and typical sclerotia were found in them. The check seedlings in the uninoculated test tubes remained alive and sterile.

On February 10 culture no. 2 was used in making similar inoculations on 26 sterile seedlings of Blue Rose rice. All the seedlings were killed by the fungus and at the end of 3 weeks conidia of a *Helminthosporium* were noted on 20 of them. Sclerotia were produced in all cases. Five uninoculated check plants remained alive and sterile.

Culture no. 3 was used on February 10 in similar inoculations on seven sterile Blue Rose rice seedlings. The *Helminthosporium* conidial stage was subsequently found on two of them. Six of the seed-

lings were killed by the fungus. Four uninoculated check plants remained alive and sterile.

On June 7, 1930, culture no. 4 was used in similar inoculations on six sterile seedlings of Fortuna rice. The inoculated seedlings were killed by the fungus but no spores were produced on any plant. Characteristic sclerotia, however, were produced in one plant and in the agar of that tube. The uninoculated check plants remained alive and sterile.

No inoculation of seedlings was made with cultures 5, 6, 7, and 8. However, cultures 5, 6, and 7 produced the typical conidial stage when transferred to corn-meal agar slants with a 4 percent agar base.

The results of these inoculations indicated that under the conditions of the experiments the strains of the stem-rot fungus from the United States, Japan, and India are similar in their ability to produce conidia of a *Helminthosporium*.

CULTURAL STUDIES

Cultural studies were made of the *Helminthosporium* obtained from the inoculations of sterile rice seedlings just described. Corn-meal-agar dilution plates were prepared with the conidia to determine whether they were viable and to study the type of germination.

On February 10, 1930, four plates were poured from the conidia produced from culture no. 2. Germination was noted 12 hours later. Germ tubes had been produced from one or both end cells of the conidia and an arborescent mycelium was developing. Forty-eight hours later the growth was visible to the naked eye. At the end of a week small white spherical bodies had developed in all the cultures. These finally matured and proved to be the typical sclerotia of *Sclerotium oryzae*, which, when transferred to corn-meal-agar slants, produced typical colonies and sclerotia. The plates were kept under observation for 1 month, but no conidia were produced in any of the cultures.

On August 1, 1930, a second set of four dilution plates was prepared with conidia from transfers of culture no. 3 made June 15, 1930. When growth was visible, 48 hours after the plates were poured, 12 single spores were located with the aid of a microscope. These were then transferred to corn-meal-agar slants. At the end of 2 weeks each of these cultures had produced sclerotia typical of *Sclerotium oryzae*. For more than 3 months these cultures were examined frequently for conidia, but none was found in any of the 12 cultures.

On December 7, 1930, another set of dilution plates was poured with conidia from the transfers of culture no. 3. These conidia were slow in germinating. On the third day, however, germination began; some of the conidia did not germinate until the fourth day. Typical growths were produced. At the end of the first week there appeared in the agar irregular dark sclerotioid bodies with coarse, rather short, dark-colored radiating hyphae. Some of the bodies were completely embedded; others were formed at the surface of the agar. The submerged bodies (fig. 1, *A*) produced conidia sparsely (fig. 1, *B*); those on the surface produced conidia abundantly (fig. 1, *C*).

Soon after the discovery of the conidia all the cultures of *Sclerotium oryzae* in the laboratory were examined. Of the 30 available tubes of cultures 1, 2, 3, and 4, *Helminthosporium* conidia were found in 9; these represented cultures 1, 2, and 3 only. Later, conidia were found also in no. 4, as well as in nos. 5 to 8.

Another set of dilution plates was poured with *Helminthosporium* conidia produced on the rice seedlings that had been inoculated in test tubes with culture no. 2, as previously described. From these

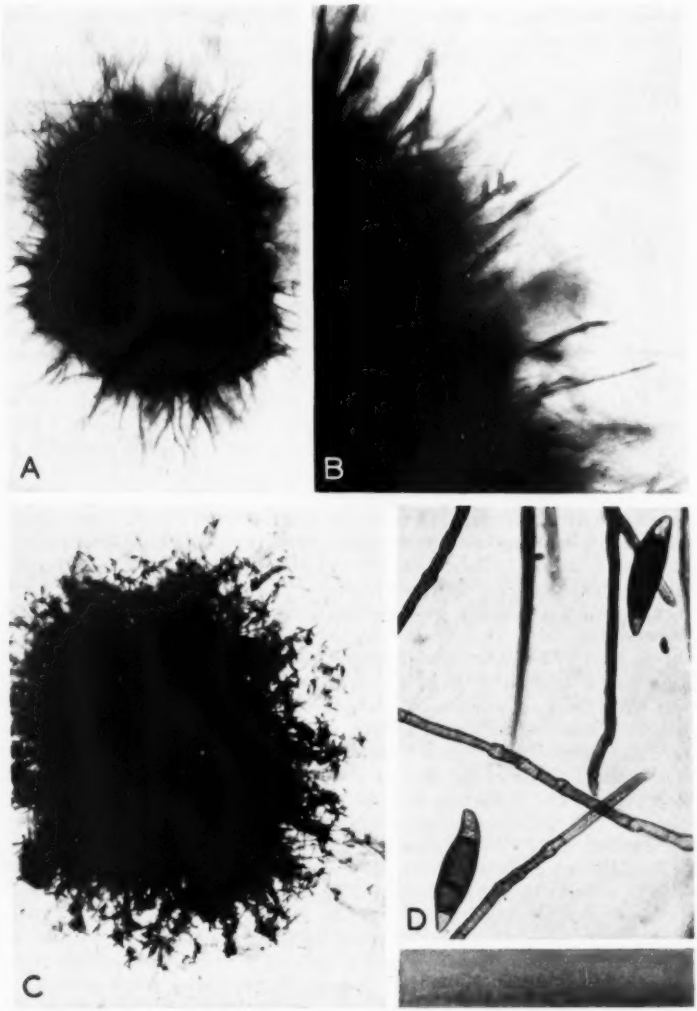

FIGURE 1.—*A*, Submerged sclerotioid body. × 25. *B*, Portion of submerged sclerotioid body showing sparse sporulation. × 60. *C*, Sclerotioid body produced on surface of agar, showing abundant conidia of *Helminthosporium sigmoideum*. × 25. *D*, Conidia and portions of conidiophores of *H. sigmoideum*. × 480.

plates 15 single-spore colonies were transferred to separate tubes of corn-meal agar. In each of the tubes sclerotia were produced but no conidia were found in any of them. Sclerotia were transferred from

these tubes to other tubes of corn-meal agar. Each of these produced sclerotia again and 11 of them also produced typical conidia of *Helminthosporium sigmoideum*.

All the cultures of *Sclerotium oryzae* produced typical spores of *Helminthosporium sigmoideum*.

INOCULATION EXPERIMENTS

With *Helminthosporium* conidia from rice seedlings that had been grown in test tubes and inoculated with *Sclerotium oryzae*, inoculations were made on individual leaves of sterile Blue Rose rice seedlings growing singly in test tubes on corn-meal agar. One leaf of each seedling was inoculated by placing on it, by means of a transfer needle loop, a small drop of sterile water in which the conidia were in suspension. Following inoculation, the seedlings were kept in diffuse light in the laboratory. After 3 days the inoculated leaves of two plants showed infection in the form of small brown elongated spots. The further development of the fungus from these spots killed the seedlings in 2 weeks. Typical *Helminthosporium* conidia were produced on the seedlings and sclerotia typical of *S. oryzae* were formed in the crown tissues of the seedlings and on the agar.

On March 21, 1930, leaves of 21 Blue Rose rice seedlings growing in soil in the laboratory were inoculated by placing a loop of a conidial suspension on each leaf. These plants were incubated for 24 hours in a saturated atmosphere at room temperature. Three days later brown lesions were observed on the leaves of 17 of the inoculated seedlings. In tissue platings made from these seedlings *Sclerotium oryzae* was obtained in only one, in which characteristic sclerotia and conidia developed. Later, microscopic examination was made of the remaining seedlings after they had been killed by the fungus, and on one additional plant typical conidia of *Helminthosporium sigmoideum* were found on sheaths just above the crown.

IDENTIFICATION

A study of the conidial stage showed that the fungus corresponded in all essential details with *Helminthosporium sigmoideum*, described and illustrated by Cavara (*5, pl. 1, fig. 5*) in 1889. Cavara's original description (*5, p. 185*) follows:

> Effusum, atrum; hyphis fertilibus sparsis erectis, rigidiusculis, hinc inde nodulosis 8–10-septatis, simplicibus, olivaceis, 100–150×5μ; gonidiis magnis, falcato-sigmoideis, utrinque obtusis, triseptatis, cellulis mediis crassioribus, granulosis, dilute olivaceis, extimis hyalinis 55–65×11–14μ.
>
> Sur les graines, les feuilles, les tiges de l'*Oryza sativa*. Environs de Pavie. Été et automne. Forme voisine du *H. hyalophloem* Sacc. (Fung. Ital. 814). Qui a cependant des hyphes fasciculées et des gonidies plus petites et point sigmoides de forme.

Cavara's illustrations are here reproduced as figure 2. Cavara (*5*) reported also the occurrence of *Sclerotium oryzae* from the vicinity of Pavia and referred to a specimen distributed in exsiccati.[3] The writer examined this specimen, in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, and found conidia of *Helminthosporium sigmoideum* as well as sclerotia of *S. oryzae*. Portions of conidiophores, also found in this specimen,

[3] BRIOSI, G., and CAVARA, F. I. FUNGHI PARASSITE DELLE PIANTI COLTIVATE OD UTILI ESSICCATI, DELINEATI E DESCRITTI. Fasc. 1, no. 25. 1888.

bore the sharp-pointed sterigmata characteristic of the conidiophores as noted by the writer (fig. 3). The conidia and conidiophores from the Briosi and Cavara specimen were found to be essentially identical with those from the writer's material.

From the cultural studies and artificial inoculations here recorded it is evident that *Helminthosporium sigmoideum* and *Sclerotium oryzae* are the conidial and sclerotial stages, respectively, of the same fungus.

LEPTOSPHAERIA SALVINII, THE ASCIGEROUS STAGE OF SCLEROTIUM ORYZAE

On some plants of Blue Rose rice and red rice grown in the greenhouse at Fayetteville, Ark., and infected with *Sclerotium oryzae*, a few peritheciumlike fruiting bodies were found on the outer sheaths. These fruiting bodies contained spores that were similar to the conidia of *Helminthosporium sigmoideum*.

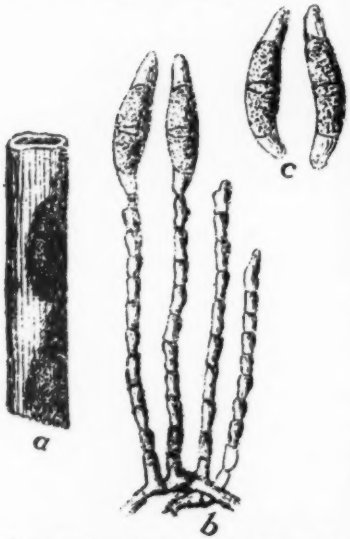

FIGURE 2.—Reproduction of Cavara's figures (*5*, *pl. 1, fig. 5*), the legend for which may be translated as follows: "*Helminthosporium sigmoideum* n. sp.; *a*, portion of stem of *Oryza* with spots caused by the fungus; *b*, fruiting hyphae; *c*, spores."

Further examination of additional specimens revealed other such fruiting bodies with the spores in asci. These asci were found in various stages of development. The fungus was identified as *Leptosphaeria salvinii*, which had been described on rice in Italy by Cattaneo (*4*) in 1879. In 1910, Miyake (*9*) reported that this fungus had not been found in Japan.

In October 1931, perithecia of *Leptosphaeria salvinii* were found on a number of rice varieties infected with *Sclerotium oryzae* at the Rice Branch Experiment Station, Stuttgart, Ark. On these perithecia, conidiophores and conidia similar to those of *Helminthosporium sigmoideum* were frequently found. This, together with the similarity of the ascospores and conidia and the fact that the perithecia were found on plants infected with *Sclerotium oryzae*, suggested the possibility that *L. salvinii* might be the ascigerous stage of *H. sigmoideum* and *S. oryzae*, which already had been shown to be connected. With this in mind, cultural studies and inoculation experiments were undertaken.

CULTURAL STUDIES

On November 17, 1931, three perithecia of *Leptosphaeria salvinii* from Blue Rose rice plants grown in the greenhouse at Fayetteville, Ark., were broken up in separate drops of distilled water to which dilute lactic acid had been added. The spore suspensions were smeared over the surface of slants of tubes of potato-dextrose agar. On the second and third days after the inoculations, 36 single-spore

colonies were located with the aid of a microscope and marked. Each colony was transferred to a separate tube of potato-dextrose agar (series 1).

On November 20 three additional perithecia were similarly broken up in distilled water to which a trace of lactic acid had been added. The drops of water were placed in sterile Petri dishes and diluted with cooled melted agar. On the second day thereafter nine transfers of single-spore colonies (series 2A) and three transfers from mycelium produced from two or more ascospores (series 2B) were made, as in the preceding experiment.

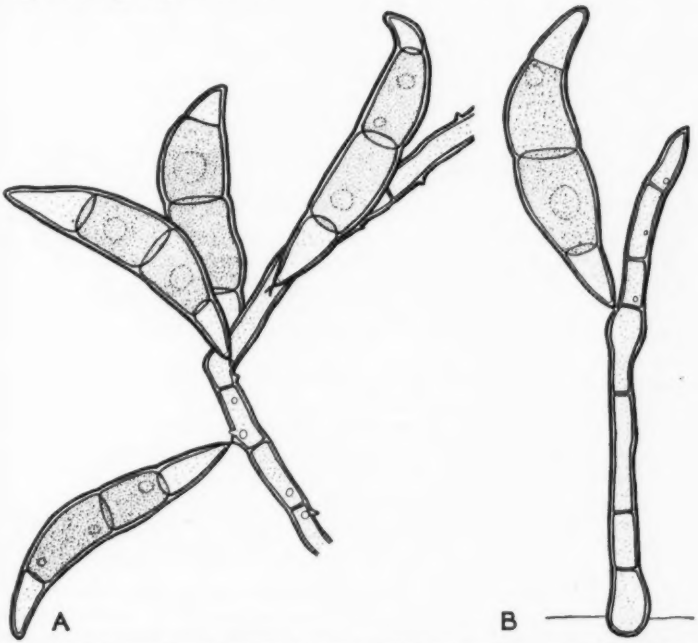

FIGURE 3.—*Helminthosporium sigmoideum*, × 1,140: *A*, Conidia and a portion of conidiophore from a test-tube culture on corn-meal agar: *B*, conidium and conidiophore from inoculated Blue Rose rice seedling grown in soil in the laboratory.

On December 4 dilution agar plates were prepared, as previously described, from perithecia from Blue Rose rice grown in the greenhouse. Three single-spore cultures were secured (series 3).

On December 9, 1931, dilution agar plates were prepared of spores from perithecia produced on red rice in the greenhouse. Three single-spore cultures were secured from these plates also (series 4).

By the last of January 1932 all of the 54 cultures of *Leptosphaeria salvinii* (series 1 to 4) had produced abundant mycelium and sclerotia typical of *Sclerotium oryzae*. On January 23, 1932, one culture from series 2A and one from series 2B produced conidia typical of *Helminthosporium sigmoideum*. By the last of February 1932, 15 of the colonies from all four series of isolations had produced conidia typical of *H. sigmoideum*.

Conidia from isolation series 1 and 2 were transferred on February 26, 1932, to corn-meal-agar slants. They had germinated by the following day, and nine single-spore colonies were located with the aid of a microscope and transferred to individual tubes. By the middle of April, five of the nine cultures had produced sclerotia typical of *Sclerotium oryzae*. No sclerotia were produced by the other four cultures. No conidia were produced in any of the nine cultures during the period that they were under observation.

Ascospores of *Leptosphaeria salvinii* from rice stubble collected at Stuttgart, Ark., intermittently from October through February germinated readily when placed in tap water at room temperature. Some of the ascospores of the October collection germinated readily the following May in distilled water at room temperature.

The characteristics of the cultures from ascospores were essentially identical with those from either conidia or sclerotia.

Numerous unsuccessful attempts have been made to produce the ascigerous stage in artificial culture, by using cultures originating from each of the three stages.

INOCULATION EXPERIMENTS

Blue Rose rice plants were grown in autoclaved virgin soil from the vicinity of Stuttgart, Ark., in three 3-gallon stoneware jars. The seeds were prepared for sowing by removing the hulls, surface-sterilizing them for 5 minutes in 1 : 1,000 mercuric chloride solution, and then rinsing them in tap water. About 10 plants were grown in each jar.

On June 21, 1932, when the plants were 6 to 8 inches high, they were inoculated with sclerotia from pure cultures originating from ascospores of *Leptosphaeria salvinii*. These cultures were obtained from stem-rotted Blue Rose and Rexoro rice plants grown at Stuttgart in 1931. No plants were left uninoculated as controls, because in numerous trials plants grown similarly without inoculation had always remained free from stem rot. Inoculation was made by placing sclerotia in contact with the plants at the water line without wounding. On August 8, 1932, all culms that had not become infected as a result of the first inoculation were reinoculated with sclerotia from the same sources as were those in the first inoculation. When the reinoculations were made the outer sheaths of a number of the plants had been killed by the stem-rot fungus as a result of the first inoculation, and the typical symptoms had been produced. Examination of the dead sheaths a week later showed mature perithecia of *L. salvinii*. In addition, large numbers of conidiophores and conidia of *Helminthosporium sigmoideum* were found on the outer sheath surfaces, and sclerotia typical of *Sclerotium oryzae* were found embedded in the tissues of the sheaths.

It is evident from the results here set forth that *Leptosphaeria salvinii* is the ascigerous stage of *Helminthosporium sigmoideum* and *Sclerotium oryzae*.

TECHNICAL DESCRIPTION OF LEPTOSPHAERIA SALVINII CATT., 1879 (*4, p. 126–127; pl. XV, figs. 1–3*)

Synonym for sclerotial stage, *Sclerotium oryzae* Catt., 1876 (*3*); synonym for conidial stage, *Helminthosporium sigmoideum* Cav., 1889 (*5*).

MYCELIUM

Hyphae white to olivaceous, septate, profusely branched, 2μ to 5μ in diameter. In culture, mycelium white at first, later smoky to black at surface of medium. On host, mycelium white inside culm, olivaceous outside. Numerous irregular olivaceous appressoria form on culm; range from 14μ to 30μ by 8μ to 24μ.

SCLEROTIAL STAGE

Sclerotia spherical or nearly so, black at maturity, surface nearly smooth, at times covered with cottony weft of white mycelium; 180μ to 280μ, mostly 230μ to 270μ. Habitat, in sheaths and culms of *Oryza sativa* (fig. 4) and *Zizaniopsis miliacea* (Michx.) Doell and Asch.

CONIDIAL STAGE

Conidiophores dark colored, septate, erect, simple or sparsely branched, 4μ to 5μ by 100μ to 175μ; conidia borne singly on sharp-pointed sterigmata, fusiform, typically three-septate, simply curved or slightly sinuous; intercalary cells Prout's brown,[4] densely granular; terminal cells lime green,[4] less granular than intercalary cells; apical cell frequently longer and less acutely pointed than basal cell; spores occasionally constricted at middle septum, 9.9μ to 14.2μ by 29μ to 49μ, mostly 11μ to 12.5μ by 34μ to 40μ. Habitat, on leaves and culms of *Oryza sativa* (fig. 3 and fig. 1, *D*) and *Zizaniopsis miliacea*.

ASCIGEROUS STAGE

Perithecia dark, globose, embedded in outer tissues of sheath, 202μ to 481μ, average diameter 381μ, beak rather short (30μ to 70μ), frequently half the diameter of perithecium in width. Beak nonprotruding, tip flush with surface of outer epidermis of sheath, visible to naked eye; asci narrowly clavate, walls almost invisible and deliquescing by the time spores mature, short-stalked, 90μ to 128μ by 12μ to 14μ, mostly 103μ to 125μ by 13.5μ; ascospores biseriate, normally eight in ascus (rarely only four), three-septate when mature, usually somewhat constricted at septae, particularly at middle septum, brown, two end cells usually lighter in color and contents less granular than middle cells, fusiform, somewhat curved, 38μ to 53μ by 7μ to 8μ, mostly 44μ to 48μ by 8μ (figs. 5 and 6). Habitat, in sheaths of *Oryza sativa*.

HOSTS AND GEOGRAPHIC DISTRIBUTION

The sclerotial and conidial stages of *Leptosphaeria salvinii* occur on all commercial varieties of rice, red rice, and *Zizaniopsis miliacea* in the rice-growing areas of Arkansas, Louisiana, and Texas. The sclerotial stage occurs in most foreign countries where rice is grown. The conidial stage is known to occur only in Italy and the United States, but no doubt it is present in the other countries where the disease occurs. The ascigerous stage has been found on seven commercial varieties of rice in Arkansas, Louisiana, and Texas, on a rice selection (C. I.[5] no. 4559) in Arkansas and Louisiana, and on commercial rice in Italy.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.
[5] C. I. denotes accession number of Division of Cereal Crops and Diseases.

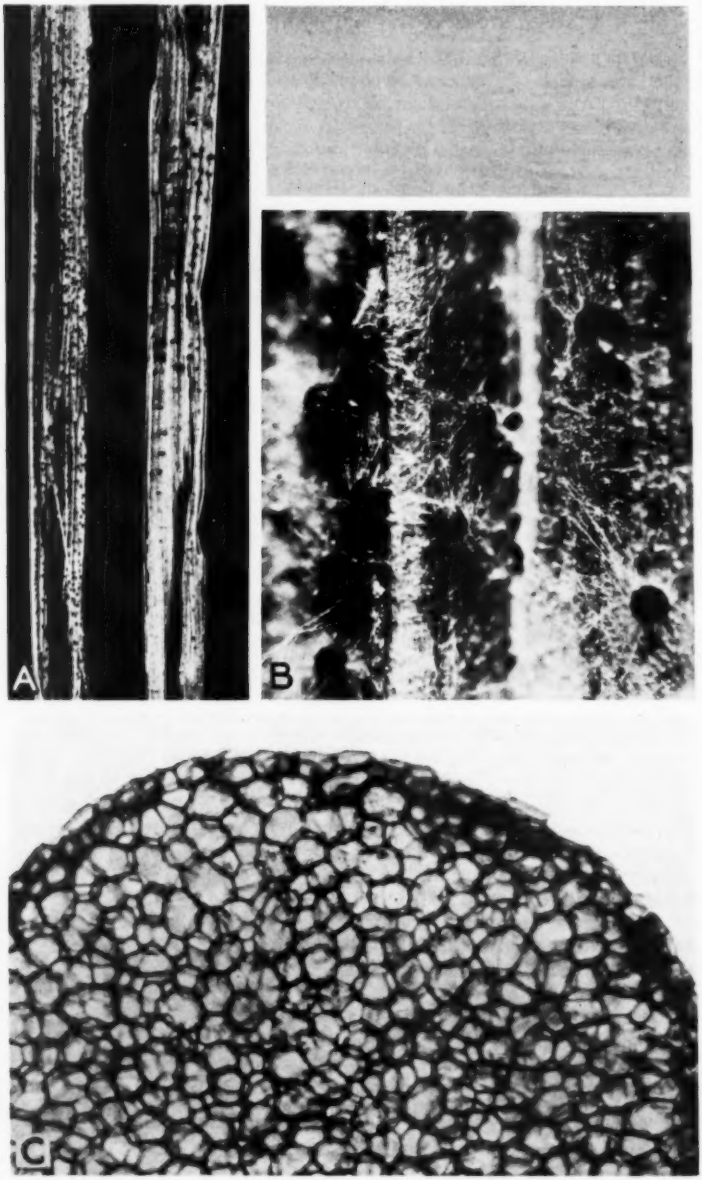

FIGURE 4.—*Leptosphaeria salvinii* sclerotia (*Sclerotium oryzae*): *A*, Sclerotia inside Blue Rose rice culm from near Beaumont, Tex. × 3. *B*, Sclerotia inside Blue Rose rice culm from near Beaumont, Tex. × 32. *C*, Portion of a cross section of a sclerotium. × 630.

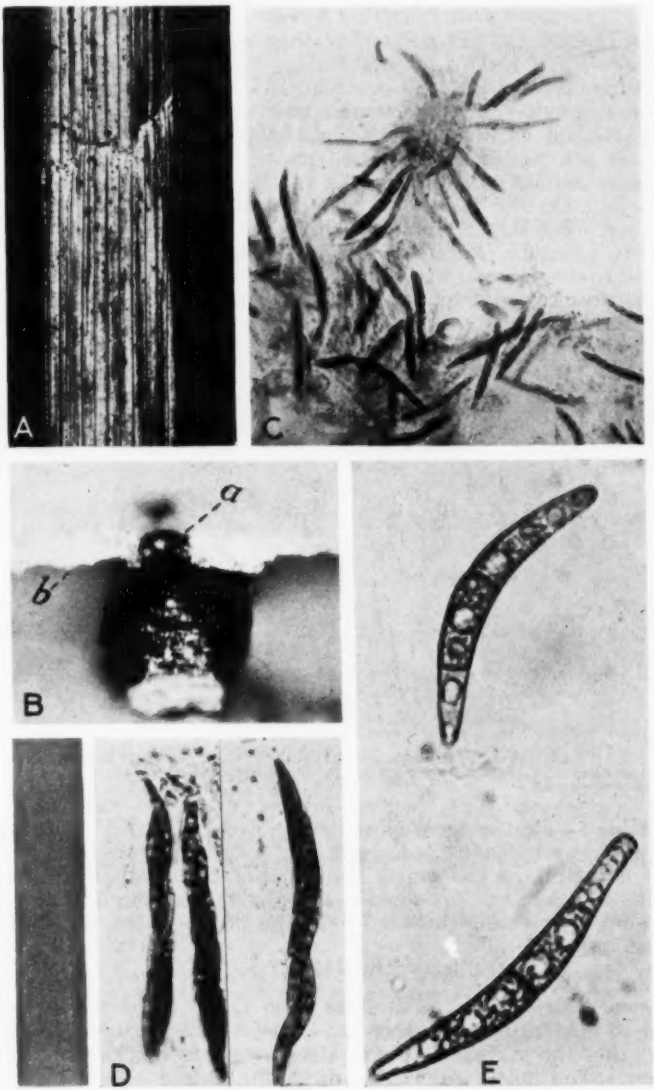

FIGURE 6.—*Leptosphaeria salvinii*, perithecial stage: *A*, Surface view of a portion of a sheath of Rexoro rice, showing the tips of the beaks of the perithecia appearing as small black specks. × 3½. *B*, Perithecium showing tip of beak (*a*) flush with epidermis of sheath (*b*). × 50. *C*, Asci in various stages of development. × 320. *D*, Asci showing biseriate arrangement o fspores. × 132. *E*, Ascopores. × 960.

SEASONAL DEVELOPMENT

As Tisdale (*18*) has pointed out, the sclerotia are known to overwinter on old rice straw and stubble in the field and to serve as inoculum for the growing rice plants during the following summer. Indications are that these overwintered sclerotia also produce abundant conidia while floating on the surface of flood water. These conidia are readily disseminated on the surface of the water and come in contact with the sheaths and there germinate and cause infections. On the infections, whether originating from sclerotia or conidia, secondary conidia develop, which serve to spread the infection still further. Sclerotia are formed in abundance in all the infected tissues later in the season. Under certain conditions the perithecia form in the infected sheaths about the same time as the sclerotia; under other conditions they appear to form somewhat earlier.

FIGURE 5.—Reproduction of Cattaneo's illustration (*4, pl. XV, figs. 1–3*), the legend for which may be translated as follows: "*Leptosphaeria salvinii* n. sp.; fig. 1, perithecium; fig. 2, *a*, ascus, *b*, spores; fig. 3, two germinating spores."

Viable ascospores have been found on old rice stubble as late in the winter as January. It has not been possible to determine whether they live through the winter and function as inoculum for the following crop because the sheaths carrying the perithecia disintegrate so completely during the winter that no perithecia have been found in the spring.

SUMMARY

Leptosphaeria salvinii Catt. has been found to be the ascigerous stage of *Sclerotium oryzae* Catt. and of *Helminthosporium sigmoideum* Cav., and the various stages of the fungus are described and illustrated. The hosts, geographic distribution, and seasonal development of the fungus are discussed.

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SOME FACTORS INVOLVED IN APHID TRANSMISSION OF THE CUCUMBER-MOSAIC VIRUS TO TOBACCO[1]

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INTRODUCTION

Evidence presented in recent years from various sources has shown that insects belonging to several different families and orders may be concerned in the dissemination of plant virus diseases. Among these, species of leaf hoppers, aphids, grasshoppers, thrips, and white fly have been incriminated as vectors of specific viruses.

The nature of the relationship between a virus and its insect vector is a matter of particular interest at the present time. The subject has already received considerable attention in connection with certain viruses transmitted by leaf hoppers, such as those of curly top of sugar beet (*19*),[2] aster yellows (*11*), and streak disease of corn (*20*), and also in connection with the potato leaf-roll virus transmitted by the green peach aphid (*6, 17, 18*). In these cases there is evidence of an intimate association between virus and insect, which appears to indicate an increase of virus within the insect's body.

Less information is available concerning the relationship involved in the transmission of typical mosaic diseases by aphids, although many virus diseases of this type are known to be disseminated by such agents. Although a certain amount of work has been done in this connection, the results so far obtained appear to be more or less fragmentary or have not been published in full.[3] A need of further information on the subject arose during recent investigations of aphid transmission of certain viruses on tobacco and related plants, some phases of which have already been reported (*7, 8*). As a foundation for critical experiments, it became necessary to determine various factors which might influence transmission, such as the number of aphids required for satisfactory infection, the duration of infectivity of the insect, and the time taken to acquire and to transmit the infective principle. Information of this type would be of value also as tending to indicate whether a biological relationship is involved between aphid and virus or whether transmission rests upon a purely mechanical basis. The cucumber-mosaic virus, which is readily transmissible to many solanaceous hosts by various species of aphids (*7, 8*), formed the subject of the present investigation, and the green peach aphid (*Myzus persicae* Sulz.) was used as the aphid vector.

[1] Received for publication Apr. 14, 1933; issued November 1933. Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U.S. Department of Agriculture.

[2] Reference is made by number (italic) to Literature Cited, p. 703.

[3] Since this manuscript was prepared, the following papers relating to this subject have been published:
BENNETT, C. W. FURTHER OBSERVATIONS AND EXPERIMENTS WITH MOSAIC DISEASES OF RASPBERRIES, BLACKBERRIES, AND DEWBERRIES. Mich. Agr. Expt. Sta. Tech. Bull. 125, 32 pp., illus. 1932.
DRAKE, C. J., TATE, H. D., and HARRIS, H. M. PRELIMINARY EXPERIMENTS WITH APHIDS AS VECTORS OF YELLOW DWARF. Iowa State Coll. Jour. Sci. 6: 347–354, illus. 1932.

REVIEW OF LITERATURE

That aphids may act as vectors of plant virus diseases was first recognized by Allard (*1*) who reported the transmission of a mosaic disease of tobacco by *Myzus persicae* and *Macrosiphum tabaci* Perg. Since this discovery a number of other virus diseases have been shown to be disseminated by one or more species of aphids. Although many reports have been limited to proof of the ability of certain species to transmit particular viruses, some have included a study of various details involved in transmission. Only the latter type of report will be considered in the present connection. The diseases concerned may perhaps be divided tentatively into two groups, namely, those of a mosaic type, such as spinach blight, cucumber mosaic, and potato mild mosaic, and those not of a true mosaic type, such as raspberry leaf curl and potato leaf roll.

McClintock and Smith (*13*), in an investigation of the transmission of spinach blight by *Myzus persicae* and *Macrosiphum solanifolii* Ashm., were able to obtain infection by using two or more aphids to a plant. According to the data presented, some infection resulted after the aphids had been allowed to remain on the diseased host for only 10 minutes, and transmission to healthy plants was effected after the aphids had remained on them for 5 minutes. Higher percentages of infection were secured, however, when longer feeding periods were allowed. These authors state that the lower instars were less infectious than the adults, that the aphids were able to infect several plants in succession, and that their infective capacity was not lost after molting. They also present evidence believed to indicate a direct transmission of the virus from parent aphid to offspring up to the fourth generation.

Doolittle and Walker (*5*), in a study of the transmission of cucumber mosaic on cucumber by *Aphis gossypii* Glov., report that the aphids were able to acquire and to transmit infection after feeding periods of 5 minutes in either case, and that they nearly always lost their infectivity after once feeding on a healthy plant. They also found that the aphids did not transmit the disease after 6 to 8 hours' confinement in a glass tube, a period said to be approximately equal to the length of time that expressed juices of diseased cucumber plants remain infectious. They state that it appears that aphis transmission of mosaic results from the virus being carried into the plant tissues on the insect's proboscis and that the minute amount of virus thus carried is exhausted during the first feeding period. So far as the writer is aware, however, these authors have not published in full the data upon which their conclusions are based.

In tests of the transmission of potato mild mosaic by *Myzus persicae*, Schultz and Folsom (*14*) obtained infection occasionally by using only 1 or 2 aphids per plant and more frequently by using larger numbers of insects. Storey and Bottomley (*21*) report a similar result in the transmission of rosette disease of peanuts by *Aphis leguminosae* Theob., single aphids only occasionally causing infection.

In a study of raspberry leaf curl transmitted by *Aphis rubiphila* Patch, F. T. Smith (*16*) found no evidence of transmission of the virus through the egg or to the living young. Bennett (*3*), in a more detailed investigation of the same disease, likewise states that the virus did not appear to be transmitted through the egg. Occasional

infection was obtained by means of single aphids, while 10 individuals were found to be as effective as 50 in causing infection. The insects became infective after 2 hours on a diseased plant and remained so up to 16 days afterward. In a later paper (*4*) this investigator states that all forms of the insect, including the winged form, were able to transmit the disease.

Elze (*6*), investigating the transmission of leaf roll of potato by *Myzus persicae*, reports an "incubation period" of the virus in the insect of 24 to 48 hours between the time of feeding on a diseased potato plant and the development of the infective capacity. The aphids retained their infectivity after remaining for 7 to 10 days on a nonsusceptible host (spinach); the virus was not lost after the insects molted and was not transmitted to the young. In the transmission of other potato virus diseases by various species of aphids neither the number of insects nor their stage of development were found to have much influence on the amount of infection secured.

K. M. Smith (*17*) likewise found no evidence of transmission of the potato leaf-roll virus from infective parent aphids to their progeny. The aphids retained their power of infection after feeding for 1 to 2 days on an immune host (cabbage), as well as after starvation for 4 to 5 days. Occasional infections were obtained by means of single aphids and maximum infection by means of 12 to 18 individuals per plant. This investigator also found evidence of an "incubation period" in the insect, which he states was not less than 54 hours under the conditions of the experiments (*18*). The aphids became infective after remaining for 6 hours on a diseased plant, though apparently not after 2 hours, and transmission of the virus was effected after 2 hours on a healthy plant. When viruliferous aphids were transferred to a fresh healthy plant every 24 to 48 hours, several, though not all, of the plants in any one series became infected.

MATERIALS AND METHODS

The cucumber-mosaic virus used in the present experiments was derived from the same source as that employed in previous investigations (*7*, *8*), being identical with Johnson's cucumber virus 1 (*10*), and no doubt the same as that studied by Doolittle and Walker (*5*). Young tobacco plants infected with this virus by means of artificial inoculation with extract from diseased plants served as sources of infection for the aphids. All transfers of aphids were made to tobacco, except in two experiments where young cucumber plants were also used.

Virus-free colonies of the peach aphid, consisting of winged and wingless viviparous females, were maintained on healthy young cabbage plants in a large insect cage. Smaller cages, such as the one shown in figure 1, *B*, were generally used for confining aphids on infected plants. These were constructed of a wooden framework and had two sides of glass and two sides and the top of cheesecloth, thus allowing fairly satisfactory light and ventilation within. In order to confine aphids to single plants, glass lamp chimneys, the tops of which were covered with cheesecloth, were placed over the plants set in 5-inch pots, and the lower edge was pushed down into the soil (fig. 1, *A*). These proved satisfactory when placed in a good diffuse light and shaded from direct sunlight. All insect cages were kept in a greenhouse at a

temperature of about 70° F. At the end of an experiment the aphids were destroyed by fumigation with nicotine dust or in some instances were removed from the plants by means of a camel's-hair brush. The plants were then set in a warmer greenhouse (about 85°) and observed for signs of infection. The tobacco plants required for the experiments were grown in the warmer greenhouse and were kept free from aphids by means of frequent fumigation.

Two methods were employed in transferring aphids from plant to plant. Whenever feasible, leaves or portions of leaves bearing the required number of aphids were removed from the infected host and transferred to a piece of paper placed on the test plant, the aphids being allowed to migrate to the new host at will. This method, which

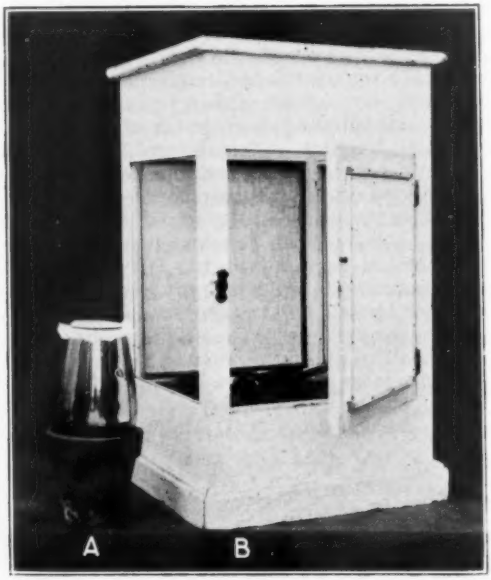

FIGURE 1.—Types of cage used for confining aphids: *A*, Lamp chimney, with cheesecloth top, enclosing a single plant; *B*, larger cage, having room for five plants in 4-inch pots

will be referred to as the "leaf method of transfer", nearly always yielded consistent and satisfactory results. It was not always applicable, however, as, for example, in experiments where the aphids were to remain for only a given length of time on a certain plant before being moved to another. In such cases the aphids were transferred by means of a camel's-hair brush slightly moistened at the tip. Particular care was exercised in handling the aphids in order to avoid injuring them in any way. Before removal they were disturbed by a light touch with the brush, which caused them to withdraw their stylets from the plant tissues. After transference, they continued to feed and to function apparently normally and showed no signs of injury. Nevertheless, for some obscure reason the brush method of transfer proved much less reliable than the leaf method. At times satisfactory infection would be secured by this method, whereas at other times,

after an identical procedure, no infection would result, even in direct transfers of aphids from diseased to healthy plants in a manner previously found to yield infection. Consequently, in experiments necessitating the use of the brush method of transfer, individual trials in which no infection was obtained throughout were not regarded as significant if subsequent repetitions of the trial under apparently similar conditions yielded definite infection, and such individual negative trials have not been recorded in the tables. On the other hand, trials that consistently yielded negative results were of course regarded as significant. As a result, the actual numbers of infections recorded in different types of experiments are not considered to be strictly comparable; they should be taken to indicate a qualitative rather than a quantitative relationship.

In experiments involving the use of the brush method of transfer a series of plants was treated as a control by touching them with the brush as if in the process of transferring aphids to or from the plants, in order to test whether infection was carried on the brush itself. When the leaf method of transfer was used, control plants were treated by placing diseased leaves free from aphids on a piece of paper resting on the plant. Each experiment was usually repeated three times, five plants being tested in each trial for each type of aphid transfer; all conclusions were therefore based on the number of infections secured on sets of 20 plants.

EXPERIMENTAL RESULTS

The first experiments of the present investigation were more or less preliminary in nature and were designed to test the effect on the infectivity of the aphids of various factors, such as the number of insects used, their stage of development, the length of feeding time on the infected host, etc. Later experiments were directed toward determining so far as possible the nature of the association between the virus and the aphid vector. Such questions as the duration of infectivity of the aphids and the time taken to develop the infective power were specially considered. The possibility of direct transmission of the virus from infective adults to their progeny was also investigated in connection with similar tests with another species of aphid reported in another paper (*9*).

INFLUENCE OF NUMBER OF APHIDS ON AMOUNT OF INFECTION

Aphids were allowed to feed for 6 days or more on tobacco plants affected with mosaic and were then transferred by the leaf method to healthy young plants under lamp chimneys, in the following numbers per plant: 1, 2, 5, 10, and 20. Transfers were made to both tobacco and cucumber, wingless adults or older nymphs being used throughout. After a week or more on these plants, the aphids were destroyed by fumigation.

When only 1 or 2 aphids were transferred to each tobacco plant, no infection was obtained; some infection resulted from the transfer of 5 aphids to each plant, and increasing percentages of infection from the transfer of 10 and 20 aphids, respectively, an average of 80 percent infection being secured by the use of 20 insects per plant (table 1). Similar results were obtained on cucumber, though on this host the incidence of infection was somewhat greater throughout, 1 and 2 aphids causing infection in single cases.

TABLE 1.—*Influence of number of aphids on amount of infection secured on tobacco and cucumber*

Number of aphids per plant	Tobacco						Cucumber			
	Number of plants infected of 5 tested in experiment no.—				Total plants infected		Number of plants infected of 5 tested in experiment no.—		Total plants infected	
	1	2	3	4			1	2		
					Number	*Percent*			*Number*	*Percent*
1	0	0	0	0	0	0	1	0	1	10
2	0	0	0	0	0	0	0	1	1	10
5	1	1	2	1	5	25	2	2	4	40
10	2	2	3	4	11	55	4	3	7	70
20	4	2	5	5	16	80	5	4	9	90
0 (control)	0	0	0	0	0	0	0	0	0	0

In order to test whether single aphids were capable of infecting tobacco, a further experiment was performed in which single individuals (wingless adults or older nymphs) were transferred from a diseased host to each of a number of tobacco and cucumber plants. Of a total of 200 tobacco plants thus tested, 13 developed mosaic; that is, an average of 1 aphid in 15 or 16 caused infection. In similar trials on cucumber, the percentage of plants infected was still higher, 14 infections being secured on 100 plants, suggesting greater susceptibility of this host to aphid infection with the cucumber-mosaic virus.

These results indicate either that every aphid does not necessarily carry infection after feeding on a diseased plant, or else that every aphid does not effect successful inoculation of the plant with the virus that it carries. Possibly both conditions obtain. Since single individuals only occasionally caused infection, it became necessary to use larger numbers in order to insure more satisfactory results, and, as a rule, 20 aphids per plant were used.

EFFECT OF CERTAIN FACTORS ON INFECTIVITY OF APHIDS

Stage of Development

In the preceding experiments only wingless adults or older nymphs were used, and it was not known whether the different forms and instars were equally effective in transmitting the virus. Comparisons were therefore made of the infective capacity of aphids in various stages of development. Since, however, it was decided to use a large number of insects per plant, it was not practicable to determine the actual instar to which each nymph belonged. The nymphs were therefore selected according to size and general appearance and classed as older, intermediate, or very young. No attempt was made to differentiate between nymphs that would develop into winged adults and those that would be wingless, although the majority were of the apterous type. Tests were made also with wingless adults. In this experiment the brush method of transfer was used, and it will be seen from table 2 that decidedly lower percentages of infection were obtained throughout, for the same number of aphids per plant, than in the preceding experiment where the leaf method was used

(table 1). Nevertheless, it is evident that all types of insect were able to transmit the disease, and no significant difference in infective capacity was observed between any of the forms tested.

TABLE 2.—*Infectivity of aphids in various stages of development*

Number of aphids per plant and developmental stage	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
	1	2	3	4		
					Number	*Percent*
20 wingless adults	0	2	3	2	7	35
20 older nymphs	3	2	2	1	8	40
20 intermediate nymphs	1	3	2	3	9	45
20 very young nymphs	3	3	0	2	8	40
0 (control)	0	0	0	0	0	0

The experiment just described showed indirectly that the aphids were able to develop the infective power after feeding on a diseased plant while they were in the nymphal stage. It did not conclusively show, however, whether they were able to acquire infectivity while in the adult stage, since the aphids tested had remained for several days on the mosaic host before transference, and the adults might have reached maturity during or after that period. In view of recent findings that the thrips vectors of "spotted wilt" of tomato and of pineapple yellowspot do not become infective if allowed to feed on diseased plants only when in the adult stage (*2, 12*), it seemed desirable to test whether a similar condition prevailed in the present connection. Wingless adults were therefore transferred by brush from the virus-free stock colony maintained on cabbage to tobacco plants affected with mosaic, and after 24 hours were removed to healthy tobacco plants, where they were allowed to remain for several days. A similar test was made at the same time with medium-size nymphs, probably of the second or third instar. Further trials were made with winged adults. On account of the relative scarcity of these in the stock colonies, however, the tests with this form were not always conducted at the same time, so that the percentages of infection obtained in the respective series are not strictly comparable. As a control, aphids from the virus-free stock colony were transferred directly to healthy tobacco plants. The results, which are presented in table 3, demonstrate that both winged and wingless adults were able to develop the infective capacity after feeding on a diseased host only in the adult stage.

TABLE 3.—*Ability of adult aphids to acquire infectivity*

Number of aphids per plant and type	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
	1	2	3	4		
					Number	*Percent*
20 wingless adults	2	4	3	3	12	60
20 winged adults	3	4	2	0	9	45
20 nymphs	2	3	4	4	13	65
Many, various types from virus-free stock colony	0	0	0	0	0	0

Feeding Periods on Diseased and Healthy Plants

In the preceding experiments, before being tested for infectivity the aphids were usually kept for several days on the host serving as the source of mosaic virus, and after transference to healthy plants remained on these for several days before being destroyed by fumigation. Tests were now made to determine the time required on the mosaic host for the aphids to acquire the infective capacity, and the time required on a healthy plant for them to transmit infection. In the first case, aphids from the stock colonies were allowed to remain for different lengths of time on mosaic plants and were then transferred by brush to healthy plants, where they remained for several days. Apterous adults and older nymphs were used, 10 aphids to each plant. Tests were made of the following periods on the mosaic host: 5 minutes, 15 minutes, 1 hour, and 24 hours. A direct transfer of aphids was made from the stock colony to healthy tobacco plants, as a test for noninfectivity of insects from this source. As shown in table 4, some infection was obtained with aphids that had remained on the mosaic host for only 5 minutes, and as much infection resulted after 15 minutes on the mosaic host as after 24 hours. The lower percentage of infection secured after the 5-minute period may no doubt be attributed, in part at least, to the fact that the aphids usually took some time to settle down on the plant and probably a certain number did not feed on the host during this short stay. It is evident, nevertheless, that they were able to become infective after a feeding period as short as 5 minutes on the mosaic host.

Table 4.—*Time required on host plants affected with mosaic for aphids to acquire infectivity*

[In each test 10 aphids were placed on each plant]

Time on mosaic host	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
	1	2	3	4		
					Number	*Percent*
5 minutes	0	2	1	1	4	20
15 minutes	4	1	2	3	10	50
1 hour	2	1	2	3	8	40
24 hours	1	4	3	1	9	45
0 (control)	0	0	0	0	0	0

In another experiment a method was adopted similar to that employed in the preceding experiment except that the aphids were allowed to remain on the mosaic host for several days and on the healthy plants for the following periods: 5 minutes, 15 minutes, 1 hour, and 24 hours. The aphids were transferred by the brush method and were removed by the same means from the test plants at the end of the experiment. Infections were obtained in all series except the controls. The average percentage of infection obtained after the 5-minute period on the healthy plants was almost as high as that obtained after the 24-hour period (table 5), indicating that the aphids were able to transmit infection to a plant almost as soon as they fed upon it.

TABLE 5.—*Time required on healthy plant for aphids to transmit infection*

Number of aphids per plant	Time on healthy plant	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
		1	2	3	4		
						Number	*Percent*
20	5 minutes	5	2	1	3	11	55
20	15 minutes	5	3	3	1	12	60
20	1 hour	3	2	3	1	9	45
20	24 hours	5	5	1	2	13	65
0 (control)		0	0	0	0	0	0

TIME REQUIRED FOR DEVELOPMENT OF INFECTIVITY OF APHIDS

The preceding experiments showed that the aphids were able to acquire and to transmit infection after very short feeding periods on mosaic and healthy plants, respectively, but gave no indication as to whether the aphids were infective immediately after first feeding on a mosaic host. Since there appears to be a delay in the development of the infective power of the insect vector of certain virus diseases, commonly termed an "incubation period" of the virus within the insect, tests were made to determine whether there was a similar period of noninfectivity of the peach aphid immediately following the first feeding period on a plant affected with cucumber mosaic.

Aphids from the virus-free stock colony were transferred to mosaic plants for 15 minutes and were then moved immediately to healthy plants for a similar period, at the end of which they were removed by means of the brush and killed. Similar tests were made in which the feeding periods were increased to 1 hour on either host, and to 1 day on the mosaic host and 3 days on the healthy plants. As a control, aphids were transferred from the virus-free stock colony to healthy plants, where they remained for 4 days. The results of these tests are presented in table 6. Infection was secured in all tests in which the aphids had first fed on a mosaic host, as much infection occurring after 15-minute periods on each host as after a total period of 4 days. Hence it is clear that the aphids were able to go through the entire process of acquiring the infective principle and transmitting infection during a period of not more than 30 minutes. It is evident that no appreciable incubation period is involved.

TABLE 6.—*Infectivity of aphids immediately following a short feeding period on a host affected with mosaic*

[In each test 20 aphids were placed on each plant]

Time on mosaic host	Time on healthy host	Number of plants infected of 5 tested in experiment no.—				Total plants infected	
		1	2	3	4		
						Number	*Percent*
15 minutes	15 minutes	3	1	3	3	10	50
1 hour	1 hour	3	2	2	2	9	45
1 day	3 days	2	2	4	2	10	50
0 (control)	4 days	0	0	0	0	0	0

DURATION OF INFECTIVITY OF APHIDS

Experiments were next conducted to determine the length of time the aphids were able to retain their power of infection. Again apterous adults or older nymphs were used and were transferred by the brush method.

Effect of Transferring Aphids to a Series of Healthy Plants

After remaining on the mosaic host for about a week, 20 aphids were transferred to each of five plants, on which they were left for 2 hours. Each group of 20 aphids was then transferred to a second plant for 2 hours, and so on, up to 4 or 5 transfers. In each test some infections were obtained on the first set of plants to which aphids were transferred from the mosaic host, a total of 10 plants out of 20 becoming diseased; but in no case were any infections secured on succeeding plants. This indicated that the aphids had lost their infectivity after 2 hours on a healthy host. Control plants remained healthy when touched with the brush as if aphids were being transferred to them from the mosaic host, indicating that infection was not carried on the brush.

It seemed possible, however, that in spite of all precautions the aphids might have become injured in some way during successive transfers, and that this might account for their failure to infect any but the first plant to which they were transferred. Accordingly, a further experiment was performed in which successive transfers of aphids to healthy plants were compared with alternate transfers to healthy and to mosaic plants. In one series, aphids were transferred from a mosaic plant to healthy plants and subsequently transferred every 24 hours to fresh healthy plants; in a second series aphids were transferred every 12 hours alternately to healthy and to mosaic plants (table 7). In the successive transfers to healthy plants, as in the preceding experiment, infections were secured only on the first set of plants tested. In the alternate transfers, on the other hand, some infections were obtained in every transfer to healthy plants. The diminishing numbers of infections obtained in succeeding transfers in this second series were probably due to the gradual loss of a certain number of aphids during the experiment. When the time came to transfer the aphids to another plant, any individuals found on the lamp chimney, or elsewhere away from the plant itself, were discarded, since it was doubtful whether these had fed on the host in question. At the end of the test, therefore, the number of aphids might be considerably reduced. The experiment indicates, nevertheless, that the loss of infective power of the aphids in successive transfers to healthy plants was probably not due to any injury sustained in handling. It might be due either to a direct loss of virus resulting from the aphid's feeding on a healthy plant, or to inactivation of the virus as a result of prolonged association with the insect. Whatever the cause of loss of infectivity, the infective capacity can clearly be reacquired by a further feeding on a mosaic plant.

TABLE 7.—*Infectivity of aphids when transferred from a host affected with mosaic to a series of healthy plants, as compared with infectivity of aphids when transferred alternately to healthy and to mosaic plants*

Experiment no.	Number of aphids per plant	Number of plants infected of 5 tested										
		Consecutive transfers [a]					Alternate transfers [b]					Controls
		1 [c]	2 [d]	3 [d]	4 [d]	5 [d]	1 [c]	2 [e]	3 [e]	4 [e]	5 [e]	
1	25	3	0	0	0	0	4	1	1	0	1	0
2	30	4	0	0	0	0	3	2	1	2	2	0
3	20	4	0	0	0	------	2	2	1	1	------	0
4	20	2	0	0	0	------	2	4	1	2	------	0
Total plants infected:												
Number	------	13	0	0	0	0	11	9	4	5	3	0
Percent	------	65	0	0	0	0	55	45	20	25	30	0

[a] Aphids transferred every 24 hours to fresh healthy plants.
[b] Aphids transferred every 12 hours alternately to healthy and to mosaic plants.
[c] Aphids transferred from mosaic to healthy plants.
[d] Aphids transferred to fresh healthy plants.
[e] Aphids transferred to fresh healthy plants after an intervening period on the mosaic host.

EFFECT OF ISOLATING APHIDS FROM LIVING HOST

In connection with the preceding experiment, it became desirable to test the length of time the aphids would remain infective when kept away from any living host. In preliminary trials, large numbers of aphids were removed by the brush method from mosaic plants and were confined in small beakers, the mouths of which were closed by means of a piece of cheesecloth held by a rubber band. After 24 hours, the aphids were returned to healthy plants upon which they were allowed to feed. As a control, other aphids were transferred by the brush directly from mosaic to healthy plants. Although several separate tests were made, no infection was obtained in any series, whether from aphids that had been confined in beakers or from those transferred directly. The absence of infection in the direct transfers from mosaic hosts, which was contrary to expectation on the basis of previous results, was attributed to the use of the brush method of transfer, already shown to yield somewhat erratic results, since later tests involving similar transfers of aphids by the leaf method yielded fairly satisfactory infection. The experiment was therefore repeated and a somewhat different procedure followed. Leaves bearing aphids were cut from the mosaic host and enclosed in beakers until a sufficient number of insects had migrated from them. The leaves were then removed and the aphids confined in the beakers for a further period before being transferred to healthy plants. Although it was not possible to determine in this way the actual time the aphids had remained away from any source of virus, this was estimated in four separate tests to be between 18 and 27 hours. No infections resulted in any trial with aphids thus treated, although the majority survived the period of starvation and appeared unharmed when returned to healthy plants. Aphids transferred directly from the mosaic host by the leaf method, on the other hand, were highly infective. It was therefore concluded that the aphids did not retain their infective capacity indefinitely, even when prevented from feeding.

Attempts to test the infectivity of aphids after shorter intervals of starvation were not very satisfactory, because of the difficulty of obtaining sufficient migration of insects from the mosaic leaves in short periods of time. However, in two separate tests, aphids which had been kept away from the living host for a period of between 2 and 3 hours were shown to be still infective. These results suggest that the loss of infectivity of aphids following a 2-hour feeding period on a healthy plant may be due to exhaustion of the supply of virus carried by the insect rather than to any inactivation of the virus as a result of contact with the aphid, though this latter factor may perhaps account for the loss of infectivity which occurred during the longer periods of starvation.

COMPARISON OF BRUSH AND LEAF METHODS OF TRANSFERRING APHIDS

The variable results secured in experiments involving the use of the brush method of transferring aphids has been a most puzzling aspect of this investigation. It has already been pointed out that the aphids showed no signs of having been injured in any way after being transferred by this means. It has also been shown that direct transfers of aphids from mosaic to healthy plants by use of the brush method yielded at times fairly satisfactory percentages of infection, according to expectations based upon the results of previous determinations, whereas at other times the amount of infection secured fell far short of that expected. There seemed some reason to believe that transmission of the virus might be influenced to some extent by the season and the prevailing environmental conditions, though whether this was due to a direct effect upon the virus, the host plant, or the insects was entirely unknown. This subject appears to deserve further investigation. On the other hand, tests involving the use of the leaf method of transfer usually yielded consistent results. Direct comparisons have therefore been made of the two methods of transferring aphids from diseased to healthy plants, and the results of four such tests are presented in table 8. In these tests the leaf method of transfer proved much more effective than the brush method for a given number of aphids, although the reason for this difference remains obscure. Unfortunately, no other suitable method of transfer has been devised which could be substituted throughout for the brush method, so that its use has perforce been continued in experiments in which other methods were not applicable.

TABLE 8.—*Comparison of the brush and leaf methods of transfer of aphids*

Experiment no.	Number of aphids per plant	Number of plants infected of 5 tested		
		Brush method	Leaf method	Control (no aphids)
1	20	2	4	0
2	20	0	3	0
3	40	2	4	0
4	40	3	5	0
Total plants infected:				
Number		7	16	0
Percent		35	80	0

"INHERITANCE" OF VIRUS BY PROGENY OF INFECTIVE APHIDS

The evidence dealing with the question of "inheritance" of plant viruses by progeny of infective insects has been discussed in some detail elsewhere (*9*). In this connection, however, it was considered worth while to test the capacity of the peach aphid for direct transmission of the cucumber-mosaic virus from infective parent aphids to their young. Aphids were accordingly reared through several generations exclusively on mosaic-diseased plants, *Nicotiana rustica* L. being used as host instead of tobacco, since this species proved more suitable for colonizing large numbers of aphids. The young nymphs were then removed at birth by means of a fine brush and transferred to healthy tobacco plants, where they were allowed to remain for about a week. Ten nymphs were transferred thus to each of 10 plants. At the same time, apterous adults were transferred similarly to another series of plants. The adults caused infection of 5 plants out of 10 tested, while all those infested with newly-born young remained healthy. In this rather limited test there was therefore no evidence of "inheritance" of the virus by progeny of infective aphids.

With a view to making a further trial by raising colonies from infective parents on some host immune to cucumber mosaic, a search was made for an immune host that would be suitable for colonizing the insects. No such plant was found, however, and the attempt was finally abandoned. Cabbage, mustard, and turnip all proved to be susceptible to infection with the cucumber-mosaic virus by means of aphids, systemic infection apparently developing when the aphids were allowed to feed freely over the plant, and only local infection when they were restricted to single leaves. This condition is similar to that already described in connection with the infection of sugar beet with the same virus (*9*). In the present instance, however, no symptoms were observed on any of the plants, although the virus could be recovered from them. Nasturtium (*Tropaeolum* sp.) and pea (*Pisum sativum* L.) also appeared to develop local infection without manifesting any symptoms. Buckwheat and celery became systemically infected, showing a conspicuous mottling of the young leaves; lettuce also proved susceptible, developing a very faint, indefinite mottling. Spinach and sugar beet have been shown elsewhere to be hosts of this virus (*9*). Many other plants are already known to be susceptible, including many members of the Cucurbitaceae and Solanaceae, and at least one species in each of the following families: Lobeliaceae, Compositae, Martyniaceae, Asclepiadaceae, Amaranthaceae, Phytolaccaceae, and Labiatae.

DISCUSSION

The results of the present investigation on the transmission of the cucumber-mosaic virus by the green peach aphid agree in most particulars with those obtained by other investigators on the transmission by aphids of virus diseases of a mosaic type. The situation involved, however, appears to be very different from that reported in connection with certain other types of virus diseases, such as curly top of sugar beet (*19*), aster yellows (*11*), and streak disease of corn (*20*), which are transmitted by leaf hoppers. The absence of an "incubation period" of the cucumber-mosaic virus in the peach aphid and the failure of the insects to retain their power of infection after feeding on a healthy plant or after starvation for 18 or more hours strongly suggest that

the association between virus and insect is in this case of a mechanical rather than biological nature. The fact that the aphids are able to complete the entire process of acquiring and transmitting infection in not more than 30 minutes further suggests that the virus is probably transferred in some manner other than in the salivary glands, for it seems doubtful whether the virus would reach these organs and be ejected from them in sufficient quantity to cause infection within such a short time after being ingested. The whole situation may be explained in a manner similar to that suggested by Doolittle and Walker (*5*), namely, that the aphid on withdrawing its stylets from a diseased host carries with it a minute quantity of infectious plant sap, which is injected into the next plant on which the aphid feeds. In this way the aphid becomes freed from the infectious material and is rendered noninfective once more. Whether such material is carried externally or within the mouth parts, however, remains undetermined. This hypothesis is somewhat similar to that of the contamination of mouth parts recently advanced by Severin (*15*) to account for the short periods of transmission of the curly-top virus by the beet leaf hopper.

The process of transmission of the cucumber-mosaic virus by the peach aphid appears to differ radically from that involved in the transmission of the potato-leaf-roll virus by the same insect (*6, 17, 18*). In the latter disease there is evidence that the virus is carried internally by the insect and that it may multiply within the insect's body, since feeding on immune hosts does not render the insect noninfective. It is significant that these two viruses may thus be associated with a single insect apparently in such different ways. It may be noted also that there are some indications of a biological relationship between the viruses of tomato spotted wilt and pineapple yellow spot and their respective thrips vectors (*2, 12*).

The conclusion that the transmission of the cucumber-mosaic virus by the peach aphid is a mechanical process has a bearing on attempts to account for the selective action exhibited by this aphid in transmitting this virus but not the true tobacco-mosaic virus from tobacco plants infected with both diseases (*7*). If transmission is accomplished merely by a transfer of infectious material upon contaminated mouth parts, two explanations of this selective action appear possible. Either, as already suggested (*8*), the tobacco-mosaic virus is not extracted from those tissues of the tobacco plant on which the aphid feeds, or else some complex interaction involving virus, aphid, and plant juice occurs which rapidly renders the virus ineffective. It must be borne in mind in this connection that certain species of aphids that do not ordinarily transmit the tobacco-mosaic virus from tobacco are able to do so from tomato (*8*). Further information appears to be needed on the distribution of the tobacco-mosaic virus both in the various tissues of the host and within individual cells.

SUMMARY

An investigation was made of the influence of various factors on the transmission of the cucumber-mosaic virus to tobacco by the green peach aphid (*Myzus persicae* Sulz.).

Single aphids were found to cause infection only occasionally, the percentage of infection increasing with the number of aphids used.

The different forms and instars appeared to be equally effective in transmitting the virus, and adults were able to develop the power of infection after feeding on a diseased host only in the adult stage. It was shown that the aphids acquired and transmitted the infective principle after very short periods on diseased and healthy hosts respectively, and that they were able to complete the entire process in a total period of not more than 30 minutes.

Viruliferous aphids were found to have lost their infectivity after a 2-hour feeding period on a healthy plant or after starvation for 18 to 27 hours. After starvation for 2 to 3 hours, however, they appeared to be still infective.

Transferring aphids by means of a camel's-hair brush was found to be a much less satisfactory method than transferring them in situ on diseased leaves and permitting them to migrate to the new host at will.

No evidence was obtained of any direct transmission of the virus by infective parent aphids to their progeny.

The results obtained are believed to indicate that the transmission of the cucumber-mosaic virus by the peach aphid is a purely mechanical process which may be explained on the hypothesis of a transfer of infectious material upon contaminated mouth parts. The process appears to be very different from that occurring in the transmission of the potato leaf-roll virus by the same insect and from that reported in connection with the transmission of certain viruses by leaf hoppers.

It is suggested that the selective action exhibited by the peach aphid in transmitting only the cucumber-mosaic virus from tobacco plants infected with a combination of this virus and the true tobacco-mosaic virus may be due to failure of the aphids to withdraw the tobacco-mosaic virus from the diseased host.

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COMPOSITION OF EGGPLANT FRUIT AT DIFFERENT STAGES OF MATURITY IN RELATION TO ITS PREPARATION AND USE AS FOOD [1]

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INTRODUCTION

To make the most effective use of the eggplant (*Solanum melongena* L.) an understanding of its physical and chemical characteristics is essential. Since the fruit of the eggplant is always eaten in the immature state, it was thought best to study its chemistry at different stages of maturity in order to determine whether it would be a more desirable food at one stage than another. An effort was made to ascertain the cause of the great shrinkage that often occurs in eggplant during cooking, as well as its tendency toward excessive softening which results in an undesirable texture or consistency. A bitterness that sometimes detracts from the quality of the product and the discoloration of material that takes place during the cooking process or when exposed to air were also studied, the table quality and the food value of different varieties were compared, and the effect of differences in stage of maturity upon the palatability of the product was determined.

REVIEW OF LITERATURE

Apparently the composition of the fruit of the eggplant has not been studied extensively. The results compiled by Chatfield and Adams (*4*) [3] show that on an average this fruit is composed of 92.7 percent water, 1.1 percent protein, 0.2 percent fat, 0.54 percent ash, and 5.5 percent total carbohydrate, including fiber; the fuel value is given as 28.2 calories per 100 g. The percentage composition as given by Van Slyke, Taylor, and Andrews (*7*) agrees very closely with the values given by Chatfield and Adams. Yoshimura (*8*) records the presence of the nitrogenous substances adenine, trigonelline, and imidazolylethylamine in the tissues of the eggplant fruit.

Several writers (*1, 3, 5*) have described methods of canning eggplant. Collections of recipes for general purposes usually describe methods of preparing and cooking the material but contain little discussion of the difficulties encountered and their causes.

SOURCE OF MATERIAL

The material used in these tests was grown at the Arlington Experiment Farm, Rosslyn, Va., in 1928. The soil was a medium heavy loam of good fertility. Frequent cultivation was given to destroy weeds and

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[2] The writers acknowledge their indebtedness to the late F. J. Pritchard, of the Bureau of Plant Industry, U.S. Department of Agriculture, at whose suggestion the work herein reported was undertaken. Mr. Pritchard supplied the material used in the tests.

[3] Reference is made by number (italic) to Literature Cited, p. 717.

to keep the soil in good tilth. The planting included the varieties New York Improved, Long Purple, Maule Excelsior, Black Beauty, Florida Highbush, Chinese Giant, Japanese Round Purple, Ovigerum, and several foreign varieties. The fruits of these varieties differed remarkably in size, shape, and color. Ovigerum has a small white egg-shaped fruit; Long Purple has a medium-sized long purple fruit; and Black Beauty and New York Improved have very large deep-purple egg-shaped fruits.

METHODS OF SAMPLING AND ANALYSIS

To secure material of known age it was necessary to know the date of flowering of each fruit. Therefore the plots were gone over every 2 days and the flowers that had opened on the preceding day or two were tagged with the record of the date. By this method the age of the fruits could be determined within a limit of error of 1 or 2 days. Practically all the samples were collected from fruits that bloomed between August 20 and September 10. The development was therefore in the cooler part of the summer, and the rate of growth was consequently slower than if earlier flowers had been tagged. Each chemical sample was made up of sections from 5 to 10 fruits, except in the case of very young samples, when 20 or more fruits were required. The samples were taken at 5- to 10-day intervals, and sampling was continued until the fruits were 60 to 70 days old. In many cases the oldest fruits were turning yellow and the seeds were brown or black, indicating complete ripeness.

The fruits were picked between 9 and 10 a.m., brought into the laboratory, and analytical samples prepared from the unpeeled fruit by slicing radial sections from each fruit so that each sample included amounts of material from the several fruits approximately proportional to their weights. The slices were then minced finely and duplicate 100-g samples were weighed out. Enough 95 percent alcohol was added to make the concentration in the preserved material 75 to 80 percent. The samples were then heated to the boiling point of the alcohol and stored until the end of the sampling period. They were then extracted with 95 percent alcohol and the extract was made up to volume. From this fraction aliquot portions were taken for determination of soluble solids, sugars, acids, tannins, and nitrate nitrogen. After extraction the residue was dried, weighed, and recorded as the alcohol-insoluble fraction. Portions of the residue were used for the determination of the acid-hydrolyzable material.

The soluble solids were determined by drying an aliquot portion of the alcoholic extract in a vacuum oven at a temperature of 75° C. The determinations were made in triplicate, porcelain milk dishes being used for the drying of the material. The value for total solids was obtained by adding the values of the soluble and insoluble fractions.

The sugars were determined by the volumetric permanganate modification of the Munson-Walker method as given in Methods of the Association of Official Agricultural Chemists (*2*).

The acids were determined by titration with N/10 sodium hydroxide and calculated as citric acid.

The total astringency was determined by the modified Procter-Loewenthal method, and the acid-hydrolyzable polysaccharides were

determined in the residue after extraction with alcohol by boiling with hydrochloric acid. Both are described in Methods of the Association of Official Agricultural Chemists (*2*).

The total nitrogen was determined by the method recommended by Ranker (*6*) for determining total nitrogen including nitrate nitrogen. For this test a separate sample was dried in a special drying chamber with a strong current of air directed against it by an electric fan.

RESULTS OF ANALYSES

Table 1 shows the results of the analyses.

SOLIDS

It is evident from the percentage of total solids that the water content of the tissues of the eggplant is high, generally between 91 and 93 percent. The percentage of total solids in the developing

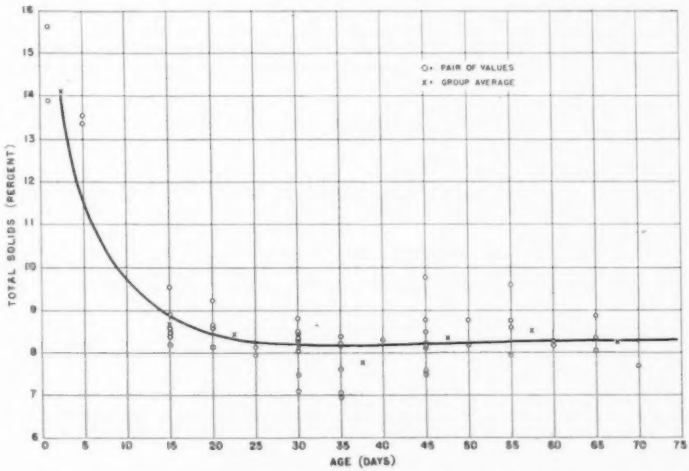

FIGURE 1.—Relation of percentage of total solids to age of eggplant fruit. Plotted from table 1; all varieties at all stages of maturity included.

fruit is largest at the time of flowering, when the values may be as high as 15 percent. They decrease rapidly for several days, but by the time the fruits are 15 to 20 days old a rather constant level has been reached. During the remainder of the developmental period there is no consistent tendency for the total solids either to increase or to decrease (fig. 1). From these results it is apparent that at any stage of maturity at which the fruits are likely to be used in cooking the difference in age will scarcely account for any significant difference in the solids content of the fruit.

In the 10 varieties studied there seem to be no consistent or significant varietal differences in solids content. There are striking differences in size, shape, and color of the fruit, but these do not seem to influence materially the moisture content.

TABLE 1.—*Composition of fruit of different varieties of eggplant at different stages of maturity*

Variety	Diameter	Age	Soluble solids	Insoluble solids	Total solids	Reducing sugar	Sucrose	Total sugar	Titratable acidity	Total astringency	Acid-hydrolyzable substances	Nitrate nitrogen	Total nitrogen	Specific gravity of flesh
	Cm	*Days*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Florida Highbush	0.6– 0.9	1	5.27	8.62	13.89	0.51	0.72	1.23	0.207	0.288	1.256	0.	0.694	
	1.0– 1.2	5	5.16	8.21	13.37			1.29	.772	1.076		0.	.602	
	4 – 5	15	4.13	4.76	8.89	1.50	.42	1.92	.240	.516	1.31	.00014	.260	0.654
	6 – 8	25	4.10	3.82	7.92	2.38	.37	2.75	.169	.202	1.10	.00026	.186	.638
	9 –11	30	4.04	3.05	7.09	2.49	.46	2.95	.137	.098	.92	.00039	.158	
	10 –12	35	4.06	3.55	7.61	2.48	.18	2.66	.133	.158	.99	.00040	.160	.681
	10 –12	45	4.26	3.87	8.13	2.48	.63	3.11	.131	.120	1.13	.00028	.149	.577
	11 –12	55	4.46	3.47	7.93	2.68	.60	3.28	.174	.118	1.12	.00017	.117	.640
New York Improved	.6– .8	1	6.61	9.01	15.62			1.22	.661	.913		0.	.721	
	1. – 1.5	5	5.26	8.28	13.54			.65	.610	1.093	2.85	0.	.595	
	3 – 4	15	3.78	4.58	8.36	.96	.40	1.36	.273	.468	.92	.00040	.412	
	4 – 6	20	3.92	4.63	8.55	1.79	.40	2.19	.208	.250	1.13	.00070	.247	
	7 – 8	30	4.14	4.32	8.46	2.19	.29	2.48	.182	.243	1.29	.00094	.211	.598
	8 –11	35	4.10	4.05	8.15	2.50	.16	2.66	.127	.120	1.21	.00091	.191	.614
	14 –16	45	4.30	3.19	7.49	2.52	.65	3.17	.116	.112	.91	.00096	.158	.580
	16 –18	55	4.76	3.97	8.73	2.48	1.09	3.57	.162	.193	1.16	.00051	.186	.658
Black Beauty	3 – 4	15	3.73	4.72	8.45	.99	.45	1.44	.260	.432	1.04	.00040		
	4 – 5	20	4.36	4.86	9.22	1.56	.57	2.13	.292	.443	1.07	.00056		
	6 – 8	25	4.26	3.85	8.11	2.12	.58	2.70	.196	.212	1.02	.00070		.626
	8 –10	30	4.80	3.67	8.47	2.88	.53	3.41	.176	.184	1.04	.00055		.612
	15 –18	45	4.20	3.37	7.57	2.65	.44	3.09	.130	.105	.90	.00155		.583
	18 –20	60	4.18	3.99	8.17	2.12	.64	2.76	.208	.126	1.06	.00197		.560
Chinese Giant	3 – 4	15	4.26	3.81	8.07	2.10	.50	2.60	.172	.204	.98	.00078		
	5 – 7	20	5.04	3.57	8.61	2.45	.64	3.09	.240	.359	1.13	.00092		
	8 –11	30	4.92	3.12	8.04	2.92	.83	3.75	.156	.081	.96	.00144		.776
	9 –11	45	4.82	3.30	8.12	2.65	.89	3.54	.140	.112	1.06	.00195		.700
	10 –11	65	4.58	3.46	8.04	2.36	.70	3.06	.205	.079	.84	.00227		
Long Purple	4 – 5	20	4.06	4.06	8.12	2.01	.69	2.70	.215	.118	1.16	.00097		
	4 – 6	30	4.48	3.82	8.30	2.66	.27	2.93	.156	.252	1.27	.00012		
	5 – 6	35	4.44	3.93	8.37	2.63	.03	2.66	.130	.272	1.24	.00023		.696
	5 – 7	45	4.16	4.04	8.20	2.46	.27	2.73	.156	.202	1.23	.00014		.659
	5 – 7	65	3.72	5.14	8.86	1.71	.77	2.48	.175	.091	1.18	.00161		
Maule Excelsior	8 –10	30	4.74	2.75	7.49	2.81	.71	3.52	.162	.126	.64	.00027		
	10 –11	35	4.48	2.45	6.93	2.67	.45	3.12	.172	.105	.65	.00048		.563
	10 –12	50	4.88	3.87	8.75	2.21	1.02	3.23	.240	.191	1.15	.00029		
Japanese Round Purple	2.5– 3.5	15	5.34	4.18	9.52	3.18	.50	3.68	.169	.239	1.21	.00057		
	5 – 7	30	5.02	3.29	8.31	3.20	.86	4.06	.120	.098	.99	.00071		
	6 – 7	40	5.34	2.95	8.29	3.31	.59	3.90	.127	.144	.90	.00195		.723
	6 – 7	45	5.22	3.55	8.77	2.84	1.15	3.99	.127	.175	1.31	.00184		
	6 – 9	55	5.48	4.11	9.59	2.91	.86	3.77	.195	.130	1.01	.00124		
	9 –10	65	3.96	4.37	8.33	1.81	.66	2.47	.223	.128	1.02	.00124		

Ronde de Chine	6 – 7	35	4.60	3.58	8.18	2.65	.37	3.02	.159	.279	.97	.00072		
	10 –12	50	4.64	3.55	8.19	2.44	.66	3.10	.130	.144	1.09	.00037		
	15 –17	70	4.70	2.99	7.69	2.86	.61	3.47	.130	.119	.78	.00121		.670
Noir Hâtive	4 – 5	35	4.44	2.61	7.05	1.60	1.15	2.75	.182	.198	.82	.00124		
	4.5– 6	45	4.46	4.03	8.49	1.63	.76	2.39	.241	.225	1.20	.00167		
	5 – 6	55	4.34	4.25	8.59	1.39	.68	2.07	.270	.215	1.27	.00153		
Ovigerum	3.5– 4.5	15	3.87	4.67	8.54	1.94	.43	2.37	.200	.160	1.55	.00067		
	5 – 6	30	3.88	4.92	8.80	1.98	.48	2.46	.195	.174	1.60	.00047		
	5.5– 6.5	45	4.44	5.31	9.75	2.04	.50	2.54	.227	.294	1.94	.00124		
	5 – 7	60	3.00	5.25	8.25	1.17	.28	1.45	.299	.119	1.31	.00115		

On an average, approximately one half of the total solids are soluble in alcohol. The differences in the proportion of soluble to insoluble solids at different ages and in the different varieties appear to be too small to be of any practical importance in relation to the cooking quality. It seems rather remarkable that the soluble materials in this fruit should be so little influenced by the ripening processes. Many of the oldest fruits in the samples were uniformly light yellow and were therefore physiologically mature or ripe, but throughout the entire period of sampling there was very little change in soluble solids.

SUGARS

Table 1 shows that the total sugars ranged mostly between 2.0 and 3.5 percent, which, though quite low as compared with those of other fruits, nevertheless made up a considerable portion of the soluble solids. There were some differences in individual samples,

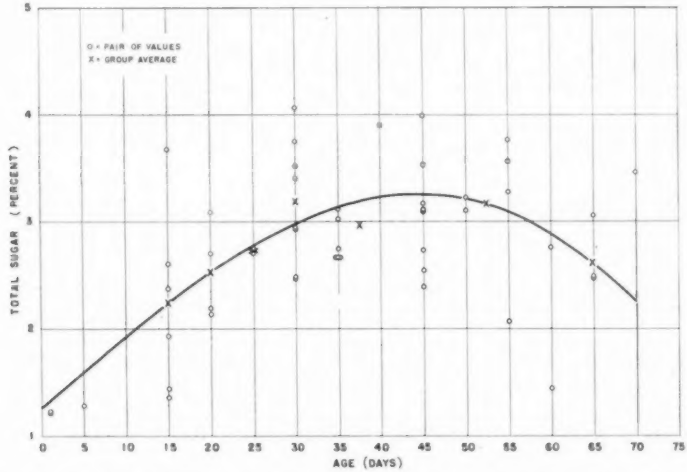

FIGURE 2.—Relation of percentage of total sugars to age of eggplant fruit. Plotted from table 1; all varieties at all stages of maturity included.

but no very marked difference in sugar content was found at any stage of maturity. There was a tendency for the sugars to increase until the fruits were 40 to 50 days old and then to decrease as they became older. This is shown in figure 2, in which the curve represents the average of all the varieties. It may be pointed out that the oldest samples of the varieties New York Improved and Florida Highbush did not decrease in sugars, but it is doubtful whether this fact is significant. The sugar content is lowest at the time of flowering, or immediately afterward, and highest when the fruits are 35 to 55 days old.

There were no very great differences in the sugar content of the different varieties; Japanese Round Purple appeared to average highest in sugar.

The principal part of the total sugars was reducing sugar, although a few samples contained as high as 1 percent of sucrose.

ACID-HYDROLYZABLE POLYSACCHARIDES

The amount of acid-hydrolyzable substances in the fruit of the eggplant was very small and there seemed to be no significant differences caused by stage of maturity or by variety.

NITROGEN

Unfortunately all the samples for the determination of total nitrogen, except those for two varieties, were lost. The total nitrogen in these two varieties indicated that the protein was very low. Total nitrogen was highest in the very young fruits, decreased rapidly for some time, then more slowly, and finally reached a constant level as the fruit approached maturity. However, the differences were too small and the number of samples too few to determine with certainty the form of the curve. These data are shown in figure 3.

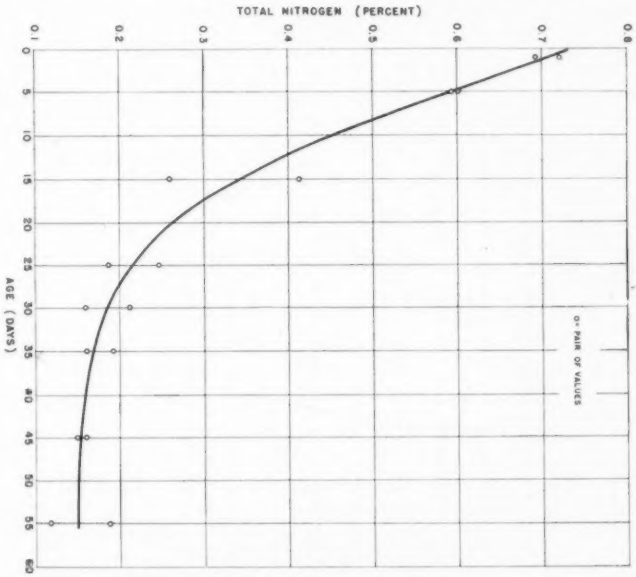

FIGURE 3.—Relation of percentage of total nitrogen to age of eggplant fruit. Plotted from table 1; values of only two varieties are included.

The percentage of nitrogen was small. If nitrates were present in the fruits at the time of flowering the quantity was too small to be measurable with the methods employed. At all ages from 15 days onward, nitrate nitrogen was invariably present and sometimes was as high as 0.0016 percent. There appeared to be no consistent differences among varieties.

SPECIFIC GRAVITY

The specific gravity was determined for a number of samples, but the results are not complete enough to indicate definitely whether there were differences due to age. It was evident, however, that

the specific gravity would be very low at any age at which the fruit would be likely to be used in cooking. The values ranged from 0.56 to 0.77, and the specific gravity of the expressed juice was 1.017, which gave a difference of 0.25 to 0.44. This indicates roughly that there was 25 to 44 percent of air by volume enclosed in the tissues. So large an amount of air in the tissues of a fruit or vegetable is rather unusual, and it might be expected that this feature would influence the behavior of the fruit in cooking.

TITRATABLE ACIDITY

The titratable acidity, calculated as citric acid, generally ranged from 0.1 to 0.2 percent in fruits 15 days old or older, but at the time of flowering a somewhat higher percentage was present. During

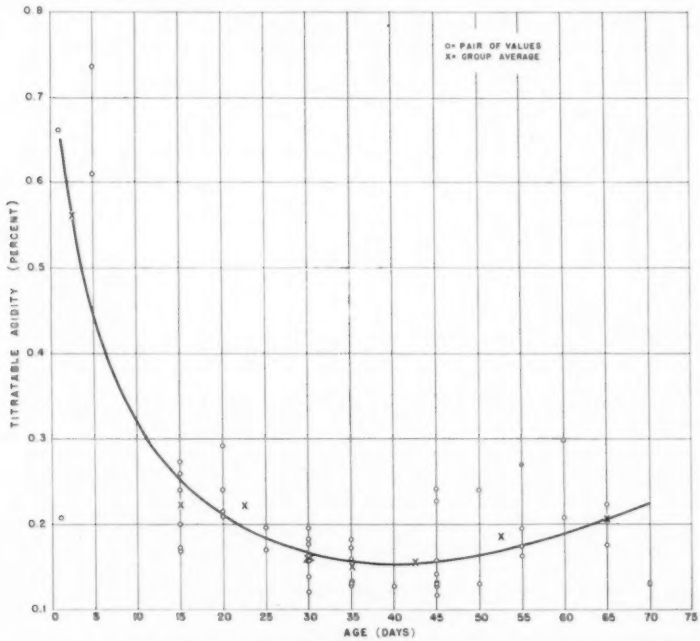

FIGURE 4.—Relation of percentage of titratable acidity to age of eggplant fruit. Plotted from table 1; all varieties included.

early development the acidity decreased slightly, reaching a low point when the fruits were 35 days of age and increasing again as they grew older. The differences, however, did not appear to be significant, at least over most of the developmental period (fig. 4).

TOTAL ASTRINGENCY

The total astringency was somewhat higher than might have been expected in view of the low total solids content. In fruits 15 days old or older total astringency in most varieties ranged from 0.15 to 0.35 percent. The total astringency was highest at the time of

flowering; for several days thereafter it decreased rapidly, then more slowly, the decrease continuing throughout the life of the fruit. There was considerable variation in individual samples, but the general trend was fairly significant. Figure 5 shows these features. The difference in total astringency among varieties seemed too small to be significant.

PECTIN CONTENT

The pectin content of the fruit was not determined quantitatively, but a number of qualitative tests were made which apparently were important. A quantity of fresh material was ground in a food chopper; the juice was pressed out immediately and strained through cheesecloth, and a portion was filtered through filter paper. The freshly expressed juice turned brown on standing in the air; it was

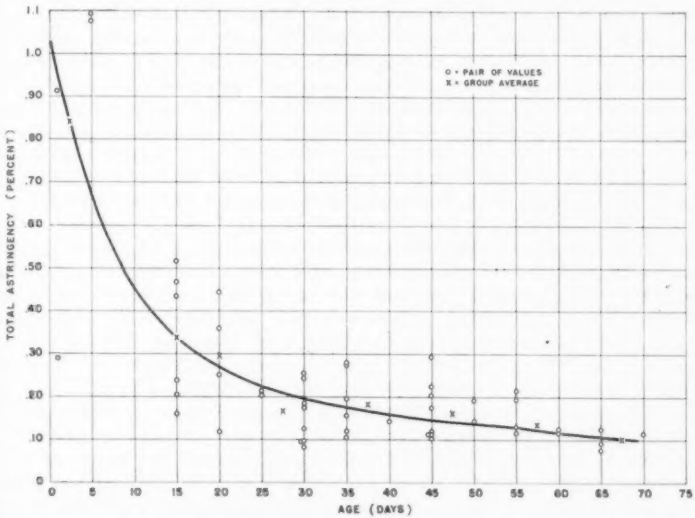

FIGURE 5.—Relation of percentage of total astringency to age of eggplant fruit. Plotted from table 1; all varieties at all stages of maturity included.

slightly viscous and gave a very slight precipitate with alcohol. A portion of the filtered juice was made alkaline with sodium hydroxide, allowed to stand overnight, then acidified with acetic acid and treated with a solution of calcium chloride. Very little precipitate was obtained. Another quantity of material was minced finely and boiled with water, forming a viscous extract which yielded a precipitate with alcohol. After this extract had been made alkaline with sodium hydroxide, allowed to stand overnight, and then acidified with acetic acid and treated with calcium chloride, the solution gave a rather heavy precipitate. Still another portion was extracted in the same way but with dilute citric acid. The extract was very viscous and could not be readily filtered through filter paper unless it was greatly diluted. The quantity of material extracted was much greater than when water was used. The extract gave a voluminous precipi-

tate with alcohol, and when treated with calcium chloride in the manner just described it gave a large amount of precipitate.

A quantity of material was boiled with dilute ammonia; this resulted in almost complete disintegration of the tissues and gave a viscous solution. Some precipitate was obtained when the solution was neutralized, and an insoluble compound was formed on treatment with calcium chloride.

These tests strongly indicate that there is a considerable amount of pectinlike material in the tissues of the fruit of the eggplant. It appears to be mostly in the form of protopectin, very little true water-soluble pectin being present.

In the course of the investigation fruits from several varieties of eggplant at various stages of maturity were steamed, boiled, or fried, in order to determine what changes take place in the cooking process. The fruits were washed, peeled, and sliced. Certain lots were salted and pressed; others were cooked without this treatment. In some recipes it is stated that salting and pressing the slices removes bitter substances. In no variety and at no stage of maturity was an objectionable bitterness found in the material used in these tests; therefore it did not seem necessary to extract the juices with salt or to soak the pieces in salt water before frying. However, the practice may have some merit, since the moisture content is very high and some of the water of the fruit may be rapidly extracted by salt or by soaking in salt water. There may be some difficulty in frying the pieces of the untreated material so that they will remain intact until served, whereas the pieces from which a part of the water has been removed will have a heavier consistency and hence a greater tendency to remain intact. The protopectin, which is largely responsible for the consistency, is not removed by the salting and pressing treatment. Of course some sugars and salts are lost, but the principal substance removed is water. If bitter substances are present they also may be partially removed. This treatment also expels a considerable portion of the air that is enclosed in the tissues.

When steamed or boiled in water the slices of the eggplant fruit became very soft and partly or wholly lost their original shape, showing considerable shrinkage. These changes were apparently due to the peculiar chemical and physical characteristics of the fruit. The softening was the result largely of the changes that occurred in the structural constituents of the tissues or to the change of protopectin into pectin as a result of heating. The high moisture content and the low starch content apparently resulted in a watery consistency which caused the material to readily lose its original shape. The shrinkage was probably due to the driving out of a large amount of the air enclosed in the tissues, which, as already stated, makes up from 25 to 44 percent of the volume of the fruit. It is evident that if water is extracted or escapes from the tissues during preparation for cooking, shrinkage results from this cause also.

The stage of maturity of the fruit seems to be of no particular importance from the standpoint of its cooking qualities. In varieties like Black Beauty and New York Improved the seeds become noticeable at the age of 35 to 40 days from the date of blooming, but even at 50 days they were not particularly objectionable. In early varieties like Ovigerum and Noir Hâtive the seeds mature considerably earlier

than in such varieties as Black Beauty and New York Improved. In these tests, fruits about two thirds grown appeared slightly superior when all points were considered. The age at which this size is reached varies with the earliness of the variety, but it was generally between 25 and 40 days under the climatic conditions under which this material was grown.

The cooking quality of the different varieties did not differ markedly and no variety seemed outstanding as compared with the others. Long Purple, Black Beauty, and New York Improved, because of the agreeable flavor and the tender quality of their flesh, were considered slightly superior to Florida Highbush and Chinese Giant. It was expected that some of the varieties would exhibit the bitterness that is cometimes complained of, but such was not the case. Since the varieties studied include those most generally grown, it seems that this trouble must be due not to the variety but to seasonal conditions or to the method of cooking. However, it must be remembered that individuals differ in their sensitiveness to unpleasant flavors. The persons judging this material were necessarily restricted to members of the writers' laboratory force. Extended tasting tests might have revealed some individuals to whom the bitterness even in this material was noticeable.

If the fruits were allowed to stand in the air after they were peeled and sliced they became somewhat brown, but the addition of salt prevented this to some extent. In practically all the cooking tests the material showed a tendency to darken, which increased somewhat when it was exposed to the air after cooking. The darkening was not sufficient to be objectionable, however, unless the material had come in contact with iron. Fruits cooked in an iron kettle became quite badly discolored. In a few tests a quantity of material that was boiled with a small amount of iron filings became greatly discolored. The discoloration was apparently due to a reaction between the iron and the tannin or other hydroxybenzene derivatives. The compounds formed promptly turn dark because of the oxidizing action of the air. The chemical analysis shows a significant amount of tannin compounds in all varieties. The low acidity also favors these reactions. The pH values of the juice ranged from 5.0 to 5.79 for the various varieties; the titratable acidity generally ranged from 0.1 to 0.2 percent. It is obvious that in order to prevent this discoloration the material must be kept from contact with iron or iron salts. The behavior of similar oxidation systems in other materials suggests that the addition of a little vinegar or lemon juice might aid in preventing any objectionable discoloration.

CANNING TESTS

No extensive canning tests were undertaken, but several lots of fruits of the varieties Black Beauty, New York Improved, Florida Highbush, and Long Purple were packed in various ways. The fruits were peeled and cut into circular disks one fourth of an inch thick and of the same diameter as the can. One lot was blanched in steam for 1 minute, packed in cans without preheating, exhausted 3 minutes, and then sealed. Another lot was prepared by thoroughly cooking the material, then pulping it and packing it while hot into the cans. In one lot the circular disks were sprinkled with salt and allowed to stand until about one third of the weight of the juice had been re-

moved; they were then packed in no. 2 cans, exhausted 3 minutes, and sealed. The juice likewise was poured into cans, exhausted 3 minutes, and sealed. All cans, regardless of their previous treatment, were processed for 1 hour at 240° F. and cooled in the air.

In several instances the product was fairly satisfactory, but on the whole the possibility of obtaining a highly attractive pack was not promising. The most satisfactory product was obtained when the material was blanched in steam and then exhausted before processing. All lots showed a tendency to darken, which seemed to be due, as already pointed out, to the presence of tanninlike compounds which reacted with the metal of the can. The reaction was favored by a very low acidity. The shrinkage was excessive unless the air was removed by pressing or exhausting. The material generally became too soft to hold its form properly. Further work would be required to develop the best method of handling the product.

There was considerable corrosion of the can similar to that caused by pumpkin. The tin was removed or dissolved, exposing the iron, which turned dark. The air or oxygen in the tissues may have been responsible for the corrosion, but the presence of nitrates may also have been an important factor. The fact that the juice alone caused practically as much corrosion as the fruit itself indicates that oxygen was probably not the only factor involved.

SUMMARY

A study was made of the composition of the fruit of the eggplant (*Solanum melongena* L.) at different stages of maturity, in relation to its behavior in cooking. The results obtained are as follows:

The total solids were low, or the moisture content high, at all stages of maturity at which the fruit would be likely to be used in cooking. The total solids generally ranged from 7 to 9 percent of the fresh green weight. They were highest in the material at the time of flowering but decreased rapidly for a few days thereafter. This decrease ceased when the fruits were 15 to 20 days old, and for the remainder of the developmental period there was usually very little change in solids.

The total sugars, which in general ranged from 2.0 to 3.5 percent of the fresh green weight, made up a substantial portion of the soluble solids. The percentage of sugar was not greatly influenced by the stage of maturity, although there was a tendency for sugar to be highest in fruit about 40 days old, or about the time when it is generally picked for table use.

The starch or other material converted into sugar by acid hydrolysis was very small in amount and differed little with stage of maturity or variety.

The percentage of total nitrogen in the material was low. It was highest in the very young fruits, decreased rather rapidly for several days, then more slowly, and appeared to reach a constant level about the time the fruits would be picked for table use. A small but significant amount of nitrate nitrogen was present at all stages of development except the very earliest.

The specific gravity of the fruits was very low; the difference in the specific gravity of the intact tissues and that of the expressed juice indicated the presence of 25 to 44 percent of air (by volume) in the tissues.

The titratable acidity was quite low at all stages of maturity. Except in the earliest stages, there was little correlation between differences in acidity and differences in age. There was a tendency for the acidity to be lowest in fruit 35 to 45 days old, or about the time when it would be used for cooking.

There was a rather significant quantity of astringent materials present in the tissues of the fruit. These were highest at the time of flowering and decreased rapidly for a few days, then more slowly until the fruit was completely mature. During the period at which the fruit would be likely to be used on the table, its age is correlated with only very small differences in the percentage of astringent materials.

The differences in the composition of the varieties studied seemed too small to be of any practical significance.

Chemical and cooking tests indicated that the failure of the material to hold its form was due to the high moisture content of the material, associated with the change of a considerable part of the protopectin into pectin during cooking. This resulted in a product that was very soft in texture; the heating had a tendency to drive out the air, causing the material to shrink.

The tendency for the material to turn dark in cooking seemed to be due to the presence of tanninlike substances associated with low acidity. Iron and salts also reacted with the tannins to form compounds that darkened in the air, hence it is obvious that care should be exercised not to bring the material into contact with iron.

In the canning process there was a tendency for the product to turn dark, become soft, and lose its form. The tin can was rather severely corroded by the material. The presence of oxygen and nitrates suggests that these may have been responsible for the action on the can.

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LOGARITHMIC EXPRESSION OF TIMBER-TREE VOLUME [1]

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INTRODUCTION

Volume tables, i.e., tabulated average volumes of trees of given diameters and heights, are basic to the management of any forest property for which the primary purpose of management is production of wood or lumber volume.

Volume tables are made for individual timber species, and often are made separately for virgin and second growth and for other intra-species classes such as those defined by site quality or by geographical location. They show cubic volume either of the entire main stem or of that part of it between the stump and a merchantable top diameter, or board-foot volume between the stump and a merchantable top diameter, this diameter being in some cases a constant and in others a variable depending upon the size of the tree.

The general method of constructing these tables is to correlate tree volume with diameter and height. Several modifications of the general method are in use. All the methods in common use imply ignorance as to the mathematical relationship of volume to dimension; for they rely upon the free-hand curve process, either entirely [3] or partially.[4] For this reason it is practically out of the question to determine the significance of differences between volume tables for two or more species in one locality or for the same species in two or more localities; the effort of fitting free-hand curves to each of the sets of data cannot be so objective but that differences result from the personal factor involved in this operation, and these differences cannot be segregated from those due to differences in species or locality.

It is the purpose of this paper (1) to present a theory bearing on the mathematical expression of tree volumes, of the power or logarithmic type, in terms of diameter breast high (d.b.h.) and height of tree; (2) to test the theory with available data; (3) to show that volume comparisons between species and localities can be placed upon a strictly objective basis; and (4) to apply the logarithmic equations to volume-table construction.

THE THEORY AND ITS TEST

(A) CUBIC VOLUME OF THE ENTIRE STEM IN TERMS OF D.B.H. AND TOTAL HEIGHT

The fundamental relationship is that of volume of the entire stem, with or without bark, to diameter breast high (d.b.h.) and total height

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[2] Particular acknowledgment is due Roy Chapman, junior forester, Southern Forest Experiment Station, for valuable assistance and criticism throughout the investigation, and to Bert R. Lexon, assistant silviculturist, Southwestern Forest and Range Experiment Station, who carried on part of the early work in the investigation. The tree-volume data used were supplied by A. C. McIntyre, of the Pennsylvania State College, by the Maryland Department of Forestry, and by several of the forest experiment stations of the U.S. Department of Agriculture.
[3] CHAPMAN, H. H., and DEMERITT, D. B. ELEMENTS OF FOREST MENSURATION. p. 202-212. Albany. 1932
[4] ———. See footnote 3, p. 216-265.
REINEKE, L. H., and BRUCE, D. AN ALINEMENT-CHART METHOD FOR PREPARING FOREST-TREE VOLUME TABLES. U.S. Dept. Agr. Tech. Bul. 304, 28 p., illus. 1932.

of tree. If the form of the stem is independent of its size, the volume equation is

$$V = D^2HC \tag{1}$$

in which V is cubic volume of the entire stem, D is d.b.h., H is total height, and C is a constant. This equation may be analyzed by the following reasoning: Since volume is to be in cubic feet, d.b.h. in inches, and total height in feet,

$$V = \frac{\pi}{4\ (144)}\ D^2HF \tag{2}$$

in which F is the cylindrical form factor used in forest mensuration. Equation (2) reduces to

$$V = D^2H(0.005454\ F) \tag{3}$$

so that C of equation (1) becomes $0.005454\ F$. Now if $F = 1$, equation (3) is simply the volume of a cylinder in cubic feet, when its diameter is measured in inches and its height in feet. But

$$F = \frac{V_T}{V_C}$$

in which V_T is the cubic volume of a tree and V_C is the cubic volume of a cylinder of the same height and diameter; so that if we assume the correlation of form factor with d.b.h. and height of a sample of trees to be zero, we are assuming from equation (3) that the cubic volume of the entire stem increases as the square of d.b.h. and directly as height. This, in fact, seldom holds. If, however, volume increases as certain other powers of d.b.h. and height, the volume equation becomes

$$V = D^{b_1}H^{b_2}C \tag{4}.$$

Taking logarithms, we have the linear form

$$\log\ V = b_1 \log\ D + b_2 \log\ H + \log\ \mathrm{C}.$$

If we now denote the logarithms of volume, d.b.h., height, and the constant term by X_1, X_2, X_3, and K, respectively, we have the familiar form of the multiple regression equation

$$X_1 = b_{12\cdot 3}X_2 + b_{13\cdot 2}X_3 + K \tag{5}$$

in which the partial regression coefficients $b_{12\cdot 3}$ and $b_{13\cdot 2}$ are the exponents of d.b.h. and height, respectively, in equation (4).

The theory presented, then, is that equation (4) is a rational expression of the relation of the cubic volume of the entire stem to d.b.h. and height; in other words, that the relation of the logarithm of volume to the logarithms of d.b.h. and height is linear. If so, the exponents and constant term may be empirically determined for a given sample by the method of least squares, and tests of the significance of the differences between two or more samples may be applied statistically.

An equation of this type was first applied to data for 264 yellow poplar trees. The calculated statistics are given in table 1. The logarithmic regression equation from these statistics—formulated according to the method given by Yule [5]—becomes

$$X_1 = 1.7924X_2 + 1.0565X_3 - 2.5220$$

and the multiple correlation coefficient $R_{1 \cdot 23} = 0.9981$. The test of the linearity of the relationship of the logarithm of volume to the logarithms of diameter and height is made by putting the equation into alinement-chart form and comparing the actual X_1 value of each tree with the estimated X_1 read from the chart. In figure 1 the result is presented graphically. The excellence of the fit establishes the linearity of the logarithmic relationship for these data.

TABLE 1.—*Means and standard deviations of the logarithms of d.b.h. inside bark, total height, and volume of wood of the entire stem, and gross correlation between the logarithms of the variables, for 264 yellow poplar trees*

Variable X	Mean M	Standard deviation σ	Correlation coefficient r
	Logarithm	*Logarithm*	
X_1=logarithm of volume	$M_1 = 0.934$	$\sigma_1 = 0.573$	$r_{12} = +0.990$
X_2=logarithm of d.b.h. inside bark	$M_2 = .885$	$\sigma_2 = .236$	$r_{13} = +.943$
X_3=logarithm of total height	$M_3 = 1.770$	$\sigma_3 = .154$	$r_{23} = +.893$

As this logarithmic equation is linear, we may immediately write the equation of the cylindrical form factor of these trees. If F is made equal to 1, equation (3) gives the volume of a cylinder of the same diameter and height as the tree; its logarithmic form is

$$\log V_C = 2 \log D + \log H - 2.2672.$$

Since

$$\log F = \log V_T - \log V_C$$

subtraction of the logarithmic cylinder-volume equation from the logarithmic tree-volume equation gives the logarithmic form of the form-factor equation. For the sample of 264 yellow poplar trees this is

$$\log F = -0.2076 \log D + 0.0565 \log H - 0.2548.$$

It is evident, therefore, that the divergence of the d.b.h. and height exponents from 2 and from 1, respectively, measures the change of form factor per unit change of dimension.

The same type of equation was fitted to data for eight other timber species, hardwood and conifer, representative of four forest regions. In every case the comparison of the data with the equation for their species served to confirm the hypothesis that the equation type is a general one. The logarithmic regression coefficient, the constant

[5] YULE, G. U. AN INTRODUCTION TO THE THEORY OF STATISTICS. Ed. 8, rev., p. 229–252. London, 1927.

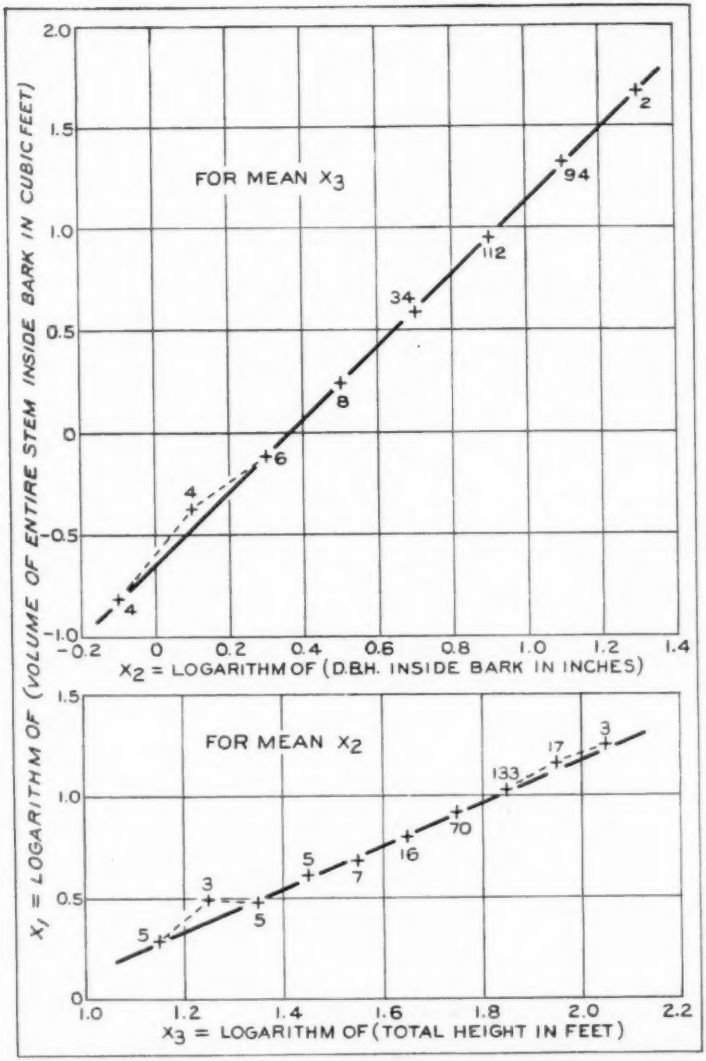

FIGURE 1.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 264 yellow poplar trees. The dependent variable is the logarithm of the cubic-foot volume of the entire stem inside bark.

term, and certain statistics for the data for each of these species are given in table 2.[6]

TABLE 2.—*Logarithmic regression coefficients, constant term, and certain pertinent statistics for nine timber species for which logarithmic equations of the cubic-foot volume of the entire stem inside bark were calculated*

Species	Partial regression coefficients		Constant, C	Means		
	$b_{12.3}$	$b_{13.2}$		M_1	M_2	M_3
				Logarithm	*Logarithm*	*Logarithm*
Virginia pine, *Pinus virginiana*	1.7023	1.2039	−2.6030	0.7708	0.5678	1.5439
Shortleaf pine, *Pinus echinata*	1.8081	1.2345	−2.7582	1.0207	.8868	1.7622
Loblolly pine, *Pinus taeda*	1.9136	.9532	−2.3723	1.2249	.9938	1.7787
Red maple, *Acer rubrum*	1.8361	1.0191	−2.4821	.4762	.6851	1.6683
Chestnut, *Castanea dentata*	1.7779	1.1302	−2.6260	.8324	.8423	1.7255
Douglas fir, *Pseudotsuga taxifolia*	1.6653	1.1800	−2.6681	2.9011	1.7038	2.3152
Black walnut, *Juglans nigra*	1.7173	1.1974	−2.6842	.7515	.8185	1.6954
Red gum, *Liquidambar styraciflua*	1.7470	1.1644	−2.6819	.9766	.9039	1.7858
Yellow poplar, *Liriodendron tulipifera*	1.7924	1.0565	−2.5220	.9340	.8852	1.7699

Species	Standard deviations			Trees, N	Standard error of estimate, $\sigma_{1.23}$	Gross correlation coefficient, r_{23}
	σ_1	σ_2	σ_3			
	Logarithm	*Logarithm*	*Logarithm*	*Number*	*Logarithm*	*Logarithm*
Virginia pine, *Pinus virginiana*	0.4117	0.1677	0.1173	846	0.0925	0.7383
Shortleaf pine, *Pinus echinata*	.5164	.1960	.1362	285	.0854	.8868
Loblolly pine, *Pinus taeda*	.5231	.2088	.1430	370	.0271	.8355
Red maple, *Acer rubrum*	.5791	.2485	.1266	537	.1570	.9072
Chestnut, *Castanea dentata*	.6729	.2761	.1651	704	.0851	.9268
Douglas fir, *Pseudotsuga taxifolia*	.4003	.1820	.0952	217	.0923	.7090
Black walnut, *Juglans nigra*	.6883	.2825	.1716	400	.0793	.9179
Red gum, *Liquidambar styraciflua*	.8693	.3334	.2460	388	.1154	.9627
Yellow poplar, *Liriodendron tulipifera*	.5731	.2362	.1539	264	.0367	.8931

In table 2 the logarithm of d.b.h. inside bark was taken as the diameter variable for every species but one. For the Douglas fir data this measurement was not available, so the logarithm of d.b.h. outside bark was used instead. The test for Douglas fir is shown in figure 2.

(B) MERCHANTABLE CUBIC VOLUME TO A FIXED TOP DIAMETER IN TERMS OF D.B.H. AND TOTAL HEIGHT

Not uncommonly an estimate of cubic volume between stump and a fixed top diameter of the stem is needed. Such a conception of cubic volume requires a modification of equation (4), which, however, does not introduce any new difficulty.

[6] It is perhaps well to call attention to methods that facilitate the use of logarithms of numbers less than 1. To evade the difficulty of using negative characteristics with positive mantissas in combination with characteristics and mantissas both of which are positive, in frequency distributions and cross-products of logarithms, two methods are available: (1) To all characteristics of that variable in which a negative characteristic appears in one or more of the variates, add a constant integer sufficient to make all characteristics positive. For instance, if the smallest volume is 0.123 cubic feet, its logarithm is $\bar{1}.0899$; in this case add 1 to all volume characteristics. The logarithmic value of this volume is then considered to be 0.0899. Adding the constant to the characteristic does not affect the standard deviation or the product-moment, but it does affect the arithmetic mean of the logarithms; the integer must be subtracted from the mean to arrive at the true mean. (2) Transcribe those logarithms that have negative characteristics to the negative form, thus:

Cubic volume=0.123	$\log \bar{1}.0899$
add zero (i.e., $+1$ ——— -1)	$+1. \quad -1$
	$0.0899-1$

and this is equal to -0.9101.

This alternative is usually the simpler procedure unless the data contain a large number of values less than 1, for no statistical constant is affected.

Let us suppose that we want an equation of cubic volume of the main stem, including bark, between a 1-foot stump and a top diameter of 4 inches. Obviously a tree must be 4 inches in d.b.h. before it contains any volume whatever by this definition; but as soon as its

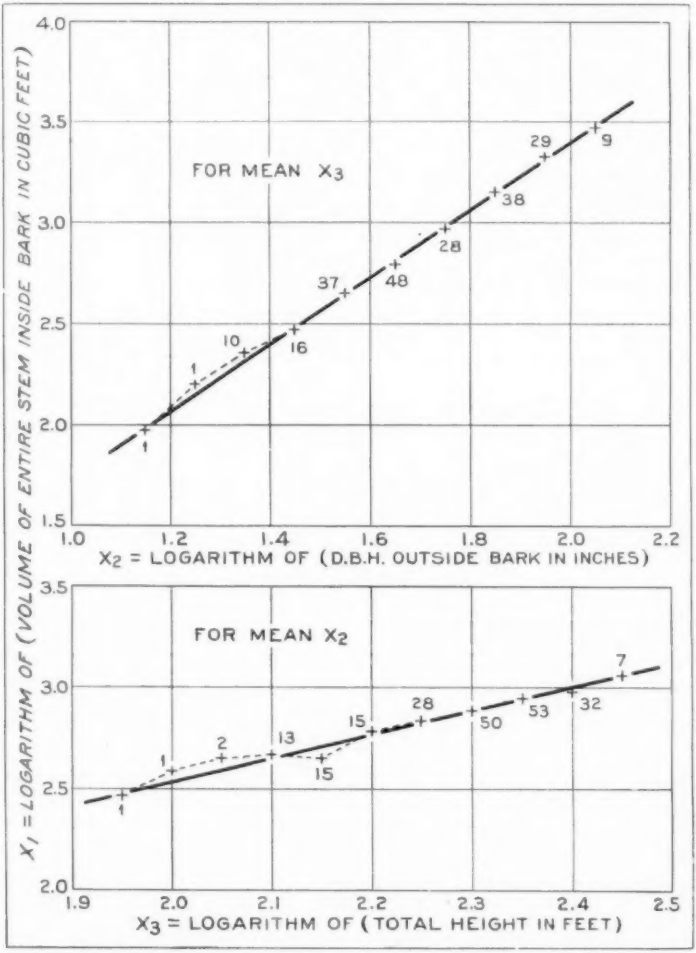
FOR MEAN X_3
X_1 = LOGARITHM OF (VOLUME OF ENTIRE STEM INSIDE BARK IN CUBIC FEET)
X_2 = LOGARITHM OF (D.B.H. OUTSIDE BARK IN INCHES)
FOR MEAN X_2
X_3 = LOGARITHM OF (TOTAL HEIGHT IN FEET)

FIGURE 2.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 217 Douglas fir trees. The dependent variable is the logarithm of the cubic-foot volume of the entire stem inside bark.

d.b.h. is just 4 inches, its volume is that of a bolt 4 inches in diameter and 3½ feet long (the length from breast height to stump). The origin of coordinates of the volume equation is therefore transferred from zero volume to the volume of this bolt (0.30 cubic foot), from zero d.b.h. to 4 inches d.b.h., and from zero height to 4.5 feet height.

Hence the equation for merchantable cubic volume corresponding to equation (4) is

$$V-v=(D-d)^{b_1}\,(H-4.5)^{b_2}C \qquad (6)$$

in which V is cubic volume of the stem between stump and the fixed top diameter d, v is the cubic volume of a bolt of diameter d and length equal to the distance from stump to breast height, and D, H, and C have the same meanings as before. Denoting the logarithms of $(V-v)$, $(D-d)$, and $(H-4.5)$ by X_1, X_2, and X_3, respectively, we have the multiple regression form of equation (5). Figure 3 shows the graphic test of the linearity of the relationship of the logarithms of the volume of the black walnut trees with the logarithms of their diameter and height, the logarithms of the volume being expressed by the equation

$$\log\,(V-0.30)=0.8274\,\log\,(D-4)+1.7262\,\log\,(H-4.5)-2.4965.$$

The merchantable volume of these trees is the cubic-foot volume in wood and bark of the stem between stump height of 1 foot and top diameter, outside bark, of 4 inches. Diameter at breast height is outside bark. The fit of the linear equation in logarithms is excellent.

(C) BOARD-FOOT VOLUME TO A FIXED TOP DIAMETER INSIDE BARK IN TERMS OF D.B.H. INSIDE BARK AND TOTAL HEIGHT

Most of the board-foot volume tables for second-growth timber list average board-foot contents between stump and a fixed top diameter inside bark, scaled according to saw-log lengths of 16 feet with the fractional log length always uppermost. Except for the change in volume unit from cubic foot to board foot, this expression of volume is the same as that of merchantable cubic volume. Equation (6) should therefore express the relationship of board-foot volume to d.b.h. and total height.

The definition of board-foot volume usually specifies, however, that a tree is considered to have board-foot volume only if its length between the stump and the fixed diameter limit amounts to at least 16 feet, that is, only if it contains at least one full saw log. Suppose we need the board-foot volume by the International log rule between a 1-foot stump and a 5-inch top diameter inside bark. Under this specification, the tree must be 5 inches in diameter inside bark at 17 feet from the ground before it is considered to have any board-foot contents. As soon as it reaches these dimensions, the origin of coordinates is transferred from zero to 5 inches d.b.h. inside bark, from zero to 17 feet height, and from zero volume to the volume of a 16-foot log 5 inches in diameter (13 board feet). Using the form of equation (6), we have

$$V-13=(D-5)^{b_1}(H-17)^{b_2}C$$

in which V is volume in board feet as defined above, D is d.b.h. inside bark, and H is total height.

This equation as it stands is misleading, in a way that is illustrated by figure 4. The two trees pictured therein are identical in d.b.h. inside bark and in total height. Tree A, containing just one full 16-foot saw log, is considered to have a volume of 13 board feet; but

tree B, containing a 13-foot saw log only, is assigned zero volume. Since the origin of coordinates is transferred to the volume of tree A,

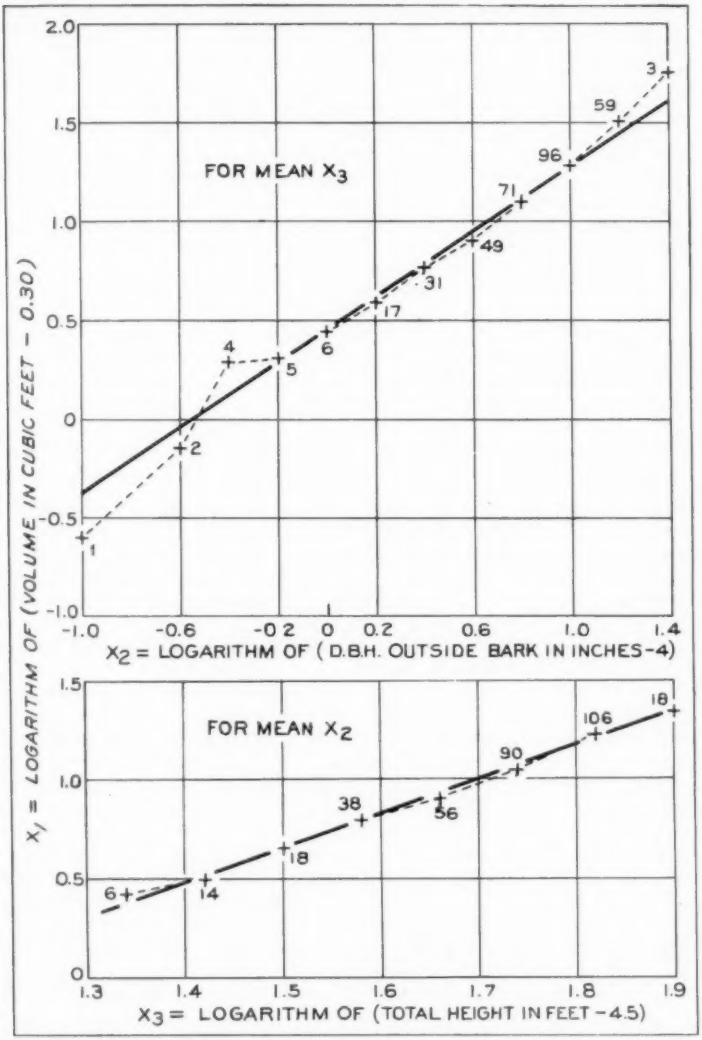
FOR MEAN X_3
X_1 = LOGARITHM OF (VOLUME IN CUBIC FEET − 0.30)
X_2 = LOGARITHM OF (D.B.H. OUTSIDE BARK IN INCHES−4)
FOR MEAN X_2
X_3 = LOGARITHM OF (TOTAL HEIGHT IN FEET − 4.5)

FIGURE 3.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 400 black walnut trees. The dependent variable is the logarithm of the cubic-foot volume to a constant top diameter less 0.30 cubic foot.

tree B must be discarded from the study, not because its independent variable, d.b.h., is below the fixed limit, but because its dependent variable, volume, is below the limit of one full saw log. Now, if obser-

vations are discarded because of restrictions placed upon the dependent variable, the regression equation is affected; for discarding the zero volumes increases the average volume of the remaining small trees, and thus lessens the regression slope of volume on diameter of these small trees. If, on the other hand, tree B is retained and zero volume assigned to it, V-13 becomes negative and its logarithm cannot be determined. The only alternative is to transfer the restriction from volume to the independent variable, d.b.h. inside bark. If this is done the regression equation is not affected. In the present instance this change is made by assigning the volume of the fractional saw log to trees that do not contain a full log, and then putting the origin of coordinates at 5 inches in d.b.h., at 4.5 feet in total height, and at $\left(\frac{3.5}{16}\times 13\right)$ board feet in volume. This treatment is more strictly in accordance with equation (6).

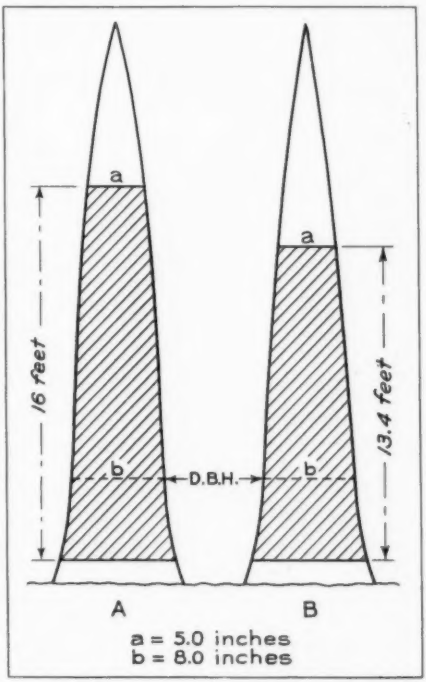

FIGURE 4.—A and B represent two trees identical in d.b.h. and in total height. Tree A contains just one full saw log of 13 board feet. Tree B contains somewhat less than a full-length saw log, and so under the usual definition of board-foot contents is assigned zero volume.

Figure 5 shows the graphic test of the linearity of the relationship of the logarithms of volume with the logarithms of diameter and height for 105 longleaf pines, which have been scaled in 16-foot saw logs by the International log rule of ⅛-inch kerf between a stump height of 1 foot and a top diameter limit of 10 inches inside bark, the logarithms of volume being expressed by the equation

$$\log\ (V\text{-}15.64) = 1.0422\ \log\ (D\text{-}10) + 0.9469\ \log\ (H\text{-}4.5) - 0.0541.$$

Figure 6 shows the same test for 275 shortleaf pines, which have been scaled in 16-foot saw logs by the Scribner log rule between a 1-foot stump and a top diameter of 8 inches inside bark, the logarithms of volume being expressed by the equation

$$\log\ (V\text{-}7.7) = 1.0512\ \log\ (D\text{-}8) + 1.2609\ \log\ (H\text{-}4.5) - 0.8023.$$

The fit of each set of data to its own equation is very good.

Attempts to substitute merchantable height for total height in volume equations of this type have so far been unsuccessful, in that the resulting logarithmic relationships have not been linear.

(D) BOARD-FOOT VOLUME TO A VARIABLE TOP DIAMETER INSIDE BARK IN TERMS OF D.B.H. INSIDE BARK AND MERCHANTABLE HEIGHT

Girard [7] has found that the actual diameter limit to which saw logs are cut from the tree tends toward a constant percentage of d.b.h. for

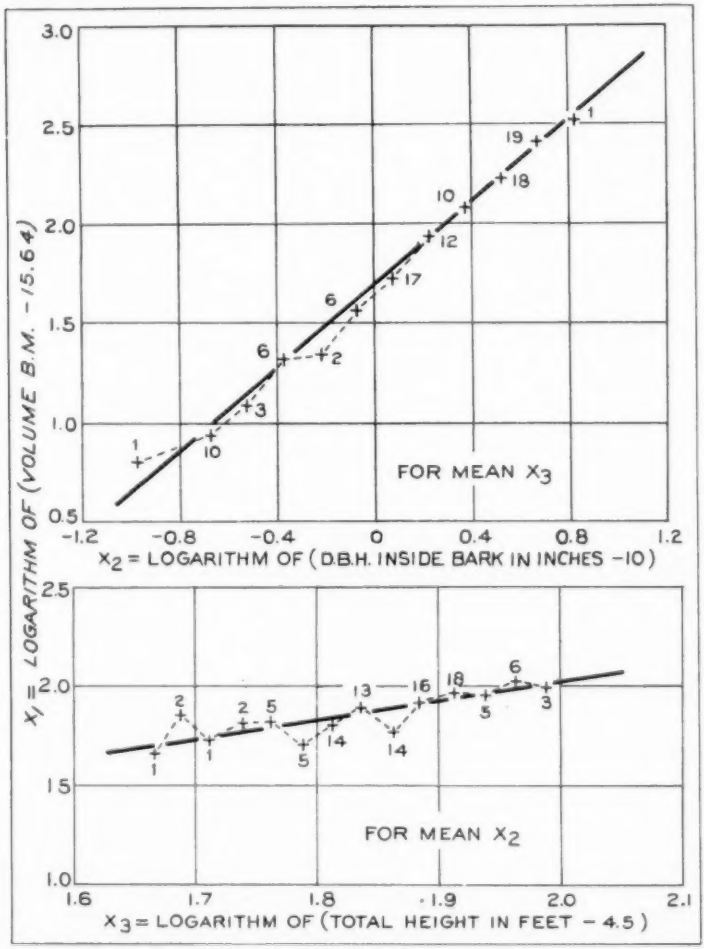

FIGURE 5.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 105 longleaf pine trees. The dependent variable is the logarithm of board-foot volume by the International log rule to a constant top diameter of 10 inches, less 15.64 board feet.

a given species. The resulting conception of board-foot volume in the tree lends itself to a logarithmic equation of the same type as equation (6), and has the advantage that the equation is linear even when the height variable is the used length rather than the total height of tree.

[7] GIRARD, J. W. VOLUME TABLES FOR MISSISSIPPI BOTTOMLAND HARDWOODS AND SOUTHERN PINES. Jour. Forestry 31: 38. 1933.

We have

$$(V\text{-}v) = (D\text{-}\tfrac{1}{2}D)^{b_1}\ (H\text{-}h)^{b_2}\ C.$$

In this case V is volume in board feet from stump to a top diameter

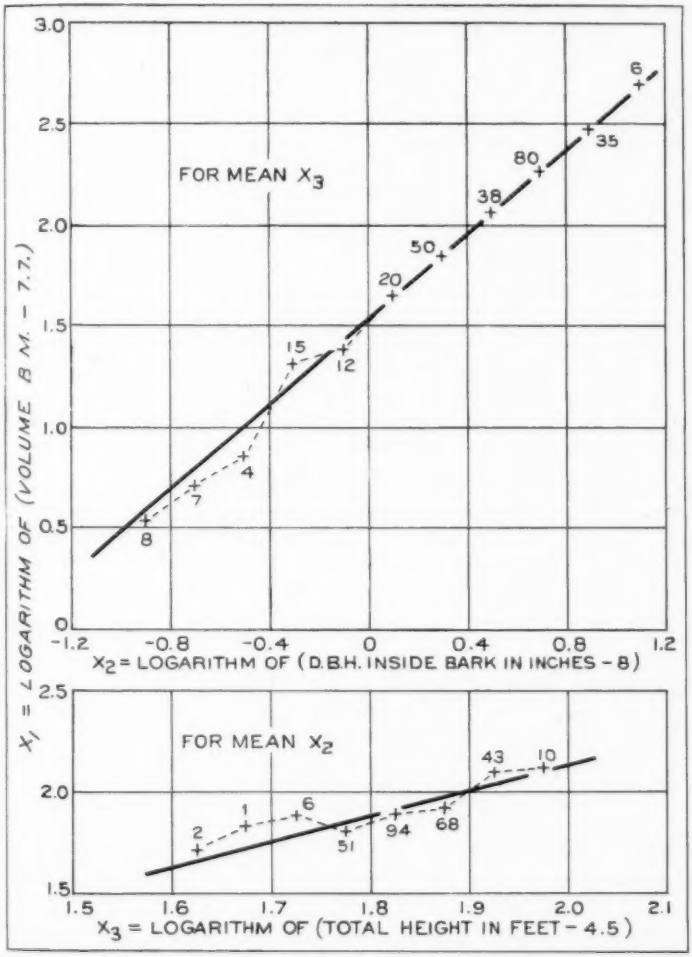
FOR MEAN X_3
X_1 = LOGARITHM OF (VOLUME B M. − 7.7.)
X_2 = LOGARITHM OF (D.B.H. INSIDE BARK IN INCHES − 8)
FOR MEAN X_2
X_3 = LOGARITHM OF (TOTAL HEIGHT IN FEET − 4.5)

FIGURE 6.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 275 shortleaf pine trees. The dependent variable is the logarithm of board-foot volume by the Scribner log rule to a constant top diameter of 8 inches, less 7.7 board feet.

equal to ½ d.b.h. inside bark, D is d.b.h. inside bark, H is height from stump to the point of $\frac{1}{2}D$, h is length from stump to breast height, and v is the board-foot volume of a log of h length and $\frac{1}{2}D$ diameter. The

equation may be somewhat simplified, for

$$(D\text{-}\tfrac{1}{2}D)^{b_1}=\frac{D^{b_1}}{2^{b_1}}.$$

The logarithmic form, then, is as follows:

$$\log\,(V-v)=b_1\log D+b_2\log\,(H-h)+C-b_1\log 2.$$

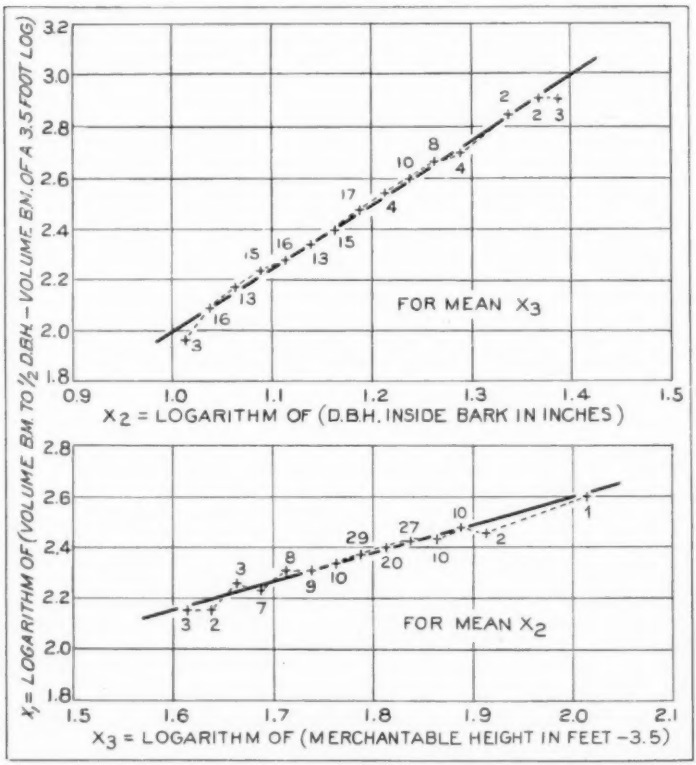

FIGURE 7.—Graphic test of the linearity of a logarithmic volume equation based on measurements of 141 loblolly pine trees. The dependent variable is the logarithm of board-foot volume by the Scribner log rule to a top diameter equal to ½ d.b.h. less the board-foot contents of a 3.5-foot log having a diameter equal to ½ d.b.h.

Since $(-b_1\log 2)$ is a constant, it is included in the C term. Figure 7 shows the graphic test of linearity of the logarithm of the board-foot contents of 141 loblolly pines, the volumes being expressed by the equation

$$\log\,(V-v)=2.4995\log D+1.1050\log\,(H-3.5)-2.4841.$$

The trees were scaled in 16-foot logs, by the Scribner rule, from stump height of 1 foot to a top diameter inside bark of ½ d.b.h. It is evident that the equation is a linear one of excellent fit.

COMPARISON BETWEEN SAMPLES OF THE SAME OR OF DIFFERENT SPECIES

Comparison of the volume equations of two different species or of two samples of the same species resolves itself into a test of the significance of the difference between the average values estimated from the two equations. The error of a particular tree's actual volume (expressed in logarithms) as compared with its estimated volume (expressed in logarithms) is, of course

$$X_1 \text{ observed} - X_1 \text{ calculated}$$

X_1 being the logarithm of the dependent variable, volume. And $\sigma_{1.23}$, the standard error of estimate of a single tree typical of the entire set, is obtained from the relationship

$$\sigma^2_{1.23} = \frac{\Sigma(X_1 \text{ observed} - X_1 \text{ calculated})^2}{N-3}$$

in which Σ is the sum of the values enclosed within the parentheses and N is the total number of trees from which the three parameters of the equation are deducted. It is, of course, not necessary to compare every observed X_1 with its calculated value in order to compute the standard error of estimate, for

$$\sigma^2_{1.23} = \frac{N\sigma^2_1(1-R^2_{1.23})}{N-3}$$

in which $R_{1.23}$ is the multiple correlation coefficient of X_1 on X_2 and X_3. The standard error of estimate of the mean X_1—i.e., M_1—of all the trees used in the regression equation is given by

$$\sigma^2_{M_1} = \frac{\sigma^2_{1.23}}{N} \qquad (7).$$

Using equation (7) we may readily test whether, for example, the mean logarithm of cubic-foot volume of the entire stem inside bark of yellow poplar is significantly different from the corresponding mean for red gum. Taking the values of M_1, $\sigma_{1.23}$, and N from table 2, we find that the two means with their corresponding standard errors are as follows:

For red gum: 0.9766 ± 0.00586.
For yellow poplar: 0.9340 ± 0.00226.

The difference between the means is 0.0426. The standard error of the difference is

$$\sigma^2_{a-b} = \sigma^2_a + \sigma^2_b \qquad (8)$$

in which σ_a and σ_b are the standard errors of the two independent values which we are comparing, namely, the means for the two species. From equation (8) the standard error of the difference between the means of the two species is found to be 0.0063. The difference is nearly seven times the error of the difference, and is therefore significant.

On the other hand, the higher M_1 of red gum is due not to a different regression equation but to the fact that the average dimensions of red gum, both d.b.h. and height, are greater. For by putting X_2 and X_3 equal to the red gum M_2 and M_3 (from table 2), respectively,

in the yellow poplar equation, we obtain the new mean

$$M_1 = 1.7924\ (0.9039) + 1.0565(1.7858) - 2.5220 = 0.9849$$

which is 0. 0083 higher than the red gum mean. Now the standard error of this estimate depends upon the average values of X_2 and X_3 used in the equation. It is the standard error of the function, i.e., of the regression equation, and is given by Miner.[8] Its value is

$$\sigma^2_f = \frac{\sigma^2_{1\cdot 23}}{N}\left(1 + \frac{(X_2 - M_2)^2}{\sigma_2^2(1-r^2_{23})} + \frac{(X_3 - M_3)^2}{\sigma_3^2(1-r^2_{23})} - \frac{2r_{23}(X_2 - M_2)(X_3 - M_3)}{\sigma_2\sigma_3(1-r_{23}{}^2)}\right) \quad (9)$$

in which σ_f is the standard error of the function. If the average values of X_2 and X_3 of the group are equal to M_2 and M_3 upon which the regression equation is based, then $\sigma_f = \sigma_{M1}$, for the terms containing X_2 and X_3 become zero.

Applying equation (9) in order to calculate the standard error of this value—the mean logarithm of the volume which yellow poplar would have if its mean d.b.h. and height variables were identical with those of red gum—we have $(X_2 - M_2) = (0.9039 - 0.8853) = 0.0186$ and $(X_3 - M_3) = (1.7858 - 1.7699) = 0.0159$, respectively. The standard error of this new mean is

$$\sigma_f = 0.00226\left(1 + \frac{(0.0186)^2}{(0.0557)(0.2026)} + \frac{(0.0159)^2}{(0.0237)(0.2026)} - \frac{2(0.8931)(0.0186)(0.0159)}{(0.0363)(0.2026)}\right)^{\frac{1}{2}} = 0.00227$$

which is so nearly the same as σ_{M1} for yellow poplar—i.e., 0.00226—because $(X_2 - M_2)$ and $(X_3 - M_3)$ are very small.

Following, then, is the comparison of the mean logarithm of volume of the two species when the mean logarithms of their d.b.h. and of their height variables are identical:

For red gum: 0.9766 ± 0.00583.
For yellow poplar: 0.9849 ± 0.00227.

The difference is 0.0083 ± 0.0063, which is not significant. Therefore, if these two samples represent their respective species in a given forest, the volumes of yellow poplar may be estimated from the red gum volume table as derived from the logarithmic volume equation for red gum. If the corresponding regression coefficients of the two species were identical, such a comparison would hold throughout the joint range of the diameter-height distributions.

In table 3 are listed the regression coefficients with their standard errors. The latter are derived from the relationships

$$\sigma_{b^2_{12\cdot 3}} = \frac{\sigma^2_{12\cdot 3}}{N\sigma^2_2(1-r^2_{23})}$$

$$\sigma_{b^2_{13\cdot 2}} = \frac{\sigma^2_{1\cdot 23}}{N\sigma^2_3(1-r^2_{23})}.$$

That the differences between the regression coefficients cannot be ascribed to differences in the characteristics of the species is apparent.

[8] MINER, J. R. THE STANDARD ERROR OF A MULTIPLE REGRESSION EQUATION. Ann. Math. Statis. 2:320-323. 1931.

It follows that if there is a region of the diameter-height distribution for which the volume estimates of the two species differ in a significant degree, this region must be at the extremities of the joint distribution. Upon comparing the calculated values of X_1 for several combinations of X_2 and X_3, we find that significant difference occurs only in the trees of small diameter, particularly those of lesser height. At $X_2=0$ and $X_3=1$, for instance, the estimated X_1 for red gum is -1.52 ± 0.021 while that for yellow poplar is -1.47 ± 0.012. The difference between these estimates is 0.05 ± 0.024, which is greater than twice its standard error. Except for very small trees, then, either equation should afford a satisfactory volume estimate for either species.

TABLE 3.—*Comparison of the logarithmic regression coefficients of yellow poplar with the corresponding coefficients of red gum*

Species	$b_{12\cdot3}$	$b_{13\cdot2}$
Yellow poplar	1.7924±0.02123	1.0565±0.03262
Red gum	1.7470± .06494	1.1644± .08801

EXPRESSION OF LOGARITHMIC EQUATIONS IN ALINEMENT CHART FORM

The logarithmic forms of the volume equations which have been discussed are equations of the first degree in three variables. Hence they may be expressed in alinement-chart form with three straight, parallel axes upon which the logarithms of the variables are graduated in arithmetic scale. A method of constructing regression equation alinement charts in general is discussed by Bruce and Reineke.[5]

Figure 8 is an alinement chart of the board-foot volume equation for shortleaf pine to a top diameter inside bark of 8 inches by the Scribner rule. The left-hand sides of the outer axes are graduated in terms of the functions of d.b.h. [$=1.0512$ log $(D-8)$] and of total height plus the constant term [$=1.2609$ log $(H-4.5)$ -0.8023], respectively; the sum of these gives the function of volume [$=$log $(V-7.7)$], and this is graduated on the left-hand side of the middle axis. A straightedge connecting any two points on the outer axes intersects the middle axis at their sum. The graduations on the right-hand side of the axes are D, H, and V corresponding to their respective functions. If the graduations of the functions are erased the volume alinement chart is in its usual form.

SUMMARY

Timber-tree volume equations of the power or logarithmic type in terms of diameter and height are deduced from known volume equations of geometrical solids on the theory that the volume of the tree stem, unlike that of the geometrical solid, may vary not directly as height and as the square of diameter, but as other powers of these dimensions in both cubic feet and board feet. The theory is tested by transforming the power equation into a linear one in logarithms and calculating the logarithmic regression equations for available

[5] BRUCE, D., and REINEKE, L. H. CORRELATION ALINEMENT CHARTS IN FOREST RESEARCH. U.S. Dept. Agr. Tech. Bul. 210, 88 p., illus. 1931.

tree data. Comparison of the actual tree volumes with those estimated from the equations confirms the hypothesis beyond practical doubt.

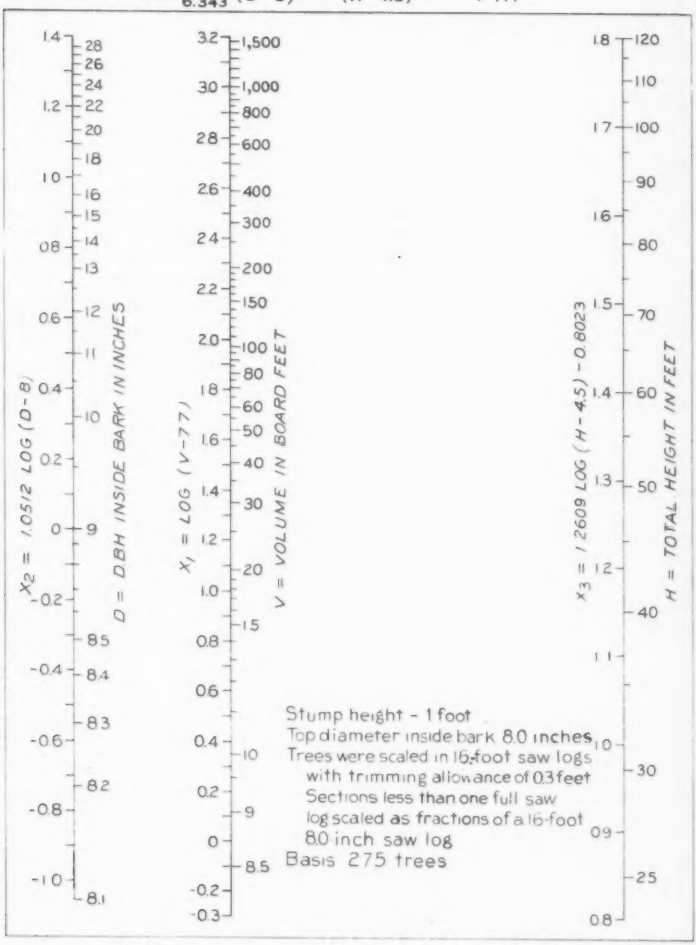
$V = \frac{1}{6.343}(D-8)^{1.0512}(H-4.5)^{1.2609} + 7.7$
$X_2 = 1.0512 \log (D-8)$
D = DBH INSIDE BARK IN INCHES
$X_1 = \log (V-77)$
V = VOLUME IN BOARD FEET
$X_3 = 1.2609 \log (H-4.5) - 0.8023$
H = TOTAL HEIGHT IN FEET
Stump height - 1 foot
Top diameter inside bark 8.0 inches
Trees were scaled in 16-foot saw logs
with trimming allowance of 0.3 feet
Sections less than one full saw
log scaled as fractions of a 16-foot
8.0 inch saw log
Basis 275 trees

FIGURE 8.—Volume alinement chart constructed from a volume equation for shortleaf pine.

The usefulness of the standard error of the regression equations in testing the significance of the difference between two sets of data, and the expression of the equations in volume alinement chart form, are illustrated.

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